

BIOAVAILABILITY OF MINERALS FROM UNREFINED CEREAL PRODUCTS

In vitro and in vivo studies

Wenche Frølich



Lund 1984



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Cand. real.

Avhandling för Fil. Doktorsexamen

Avhandlingen kommer att försvaras
vid en offentlig disputation
fredagen den 14 december 1984
kl 14.00, sal D, Kemicentrum, Lund

Organization LUND UNIVERSITY Dept. of Food Chemistry Chemical Center, P.O.Box 124, S-221 00 Lund, Sweden	Document name DOCTORAL DISSERTATION			
	Date of issue 14th December, 1984			
	CODEN:	LUTKDM/TKLK-1003/1-150 (1984)		
Author(s) Wenche Frølich	Sponsoring organization			
Title and subtitle Bioavailability of minerals from unrefined cereal products. <u>In vitro</u> and <u>in vivo</u> studies.				
Abstract The dietary fiber content in flour and cereal products on the market in Norway was investigated by using an enzymatic, gravimetric method. The variation could be explained by the different origin of the grain, but also by the variation in the extraction rate. Dietary fiber could be used as a parameter for determining the content of whole grain flour in processed products. Labeling of cereal products is necessary to the consumer when choosing products with a high dietary fiber content, as there is a very variable content of whole grain flour in the cereal products on the market. In bread with the same trade name, but produced in different bakeries, a variation in the content of whole grain flour from 20 - 65 % was observed, with a content of 45 % being most frequent. This is a clear tendency towards a higher content of whole grain flour compared with 3 - 4 years ago. A high content of whole grain flour gave, in a sensory analysis, a high score for typical bread flavor and total impression of the bread. In an <u>in vitro</u> study, the mineral binding to the dietary fiber components and breakdown of phytic acid during fermentation of bread was investigated. The different minerals studied (Fe, Zn, P, Ca, Mg) behaved differently concerning binding to the different fiber fractions before and during fermentation. The fraction of ash associated to insoluble fiber components was small and not influenced by fermentation, while there was a decrease in the minerals associated to the soluble fiber fraction, where also most of the phytate acid was found. Only 10 % of the total Fe present in the bread dough seemed to be associated to phytic acid, while 60 % seemed to be associated to fiber components, neither insoluble (40 %) nor soluble (20 %) being affected by fermentation. 61 % of the total Ca and 24 % of the Zn seemed to be associated to phytic acid as their release during fermentation ran parallel with the phytate hydrolysis. Only a small part of the total Mg was associated to fiber components or phytic acid (9 % to soluble and 3 % to insoluble). Up to 75 % of the phytic acid was recovered with the soluble fiber, and was broken down during fermentation. All phosphorus present in this fraction seemed to be present as phytate. In a long-term study using hemoglobin regeneration techniques on growing piglets, bran did not seem to have any inhibitory effect on iron absorption, even with a relatively high amount of bran (20 %). The naturally occurring iron in cereals seems to be as available as ferrous sulphate in pigs repleting from an anemic state. The influence of bran and guar-gum-enriched bread on mineral absorption was investigated in a long-term study of diabetic patients. No changes could be observed in the serum concentrations of Ca, inorganic phosphate, Mg, Fe, Cu, Zn, and Se. The urinary excretion of Ca, however, was slightly lowered during the bran period, whereas other minerals studied were not affected. This study indicates that unrefined cereal products are good sources of minerals, in spite of the presence of fiber components and phytic acid.				
Key words Cereal products, dietary fiber content, sensory properties, dietary fiber determination, mineral association, phytic acid, fermentation, mineral bioavailability, pigs, diabetic patients				
Classification system and/or index terms (if any)				
Supplementary bibliographical information		Language English		
ISSN and key title		ISBN		
Recipient's notes	Number of pages	Price		
	Security classification			

Distribution by (name and address) Wenche Frølich, Dept. of Food Chemistry, Chemical Center, University of Lund, P.O.Box 124, S-221 00 Lund, Sweden

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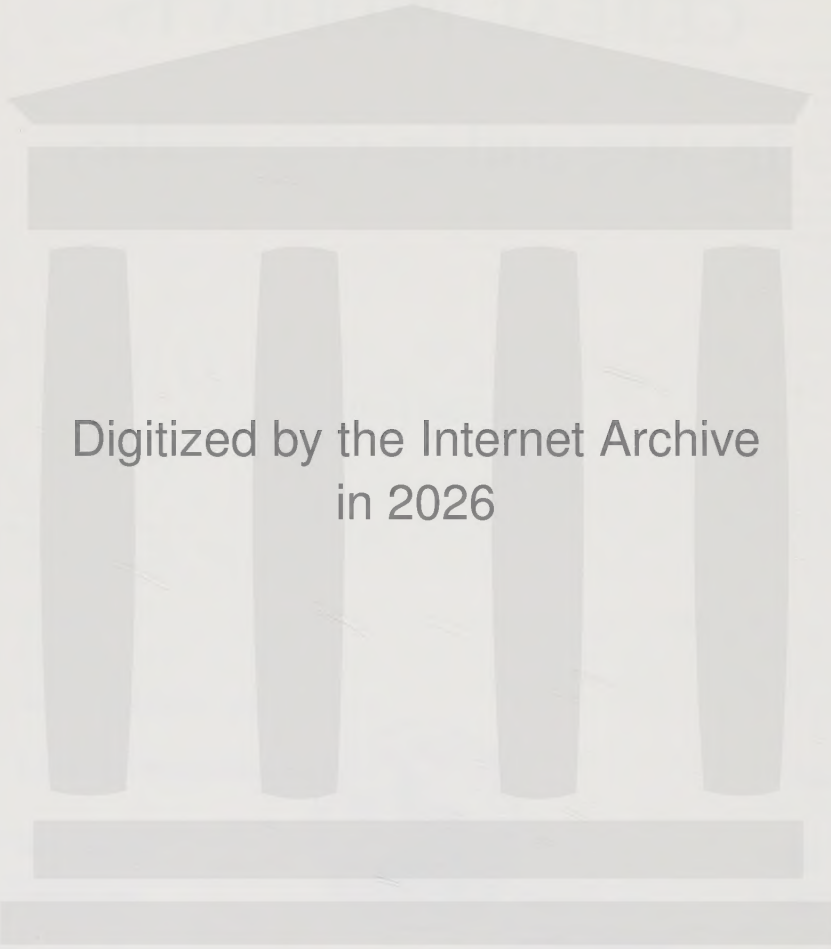
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When the path finishes,
one must really go on.
Tear the branches aside
and go out into the world.

(Stein Mehren)

PAPERS

This thesis is based on the following papers:

1. W. Frølich, N.-G. Asp, Dietary Fiber Content of Cereals in Norway.
Cereal Chem. 1981, 58, 524-527
2. W. Frølich, B. Hestangen, Dietary Fiber Content of Different Cereal Products in Norway.
Cereal Chem. 1982, 60, 82-83
3. G. Rognerud, B. Wilsher, A.M. Øybø, W. Frølich, Whole grain flour content and sensory properties in one variety of Norwegian bread.
Submitted for publication 1984
4. T.F. Schweizer, W. Frølich, S. Del Vedovo, R. Besson, Minerals and Phytate in the Analysis of Dietary Fiber from Cereals. Part I.
Cereal Chem. 1984, 61, 116-119
5. W. Frølich, T.F. Schweizer, N.-G. Asp, Minerals and Phytate in the Analysis of Dietary Fiber from Cereals. Part II.
Cereal Chem. 1984, 61, 357-359
6. W. Frølich, N.-G. Asp, Minerals and Phytate in the Analysis of Dietary Fiber from Cereals During the Process of Breadmaking. Part III.
Cereal Chem. Submitted for publication 1984
7. W. Frølich, A. Lysø, Bioavailability of iron from wheat bran in pigs.
Am. J. Clin. Nutr. 1983, 37, 31-36
8. S. Vaaler, J. Aaseth, K.F. Hanssen, K. Dahl-Jørgensen, W. Frølich, B. Ødegaard, Ø. Agenäs, Trace elements in serum and urine of diabetes patients given bread enriched with wheat bran or guar gum. Proceedings from TEMA 5, Fifth International Symposium on Trace Element Metabolism in Man and Animals, 29/6 - 4/7 - 1984, University of Aberdeen, Scotland

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INTRODUCTION

Whole grain cereals and products of these constitute some of the best sources not only of dietary fiber, but also of minerals, trace elements and B-vitamins. An increase in the intake of whole grain flour is therefore a nutritional aim in many countries.

It has, however, been claimed that mineral bioavailability is reduced in diets rich in whole grain flour products. This has been attributed mainly to the phytic acid associated with the dietary fiber, but also to the dietary fiber components themselves forming complexes with minerals and trace elements, and in this way making them less available for absorption. Due to this, the significance of whole grain flour as a source of minerals and trace elements has been questioned.

In Norway refined white flour is not enriched with minerals and trace elements. One of the best ways of increasing the diet content of minerals, trace elements and B-vitamins is via an increase in whole grain flour intake. This has been pointed out in two White Papers issued by the Norwegian Government to Parliament (no 32: "On the Norwegian Nutrition and Food Policy" from 1975 - 1976, and no 11: "On the follow-up of Norwegian Nutrition Policy", from 1981 - 1982).

If the Norwegian authorities recommend an increased consumption of whole grain flour as a means of elevating the levels of minerals and trace elements in the diet, it is therefore important to investigate the significance of the possibly decreased bioavailability of minerals and trace elements from whole grain cereals.

If the nutritional quality of bread and other cereal products on the market is to be improved, it is also important to bear in mind that this can only be done in cooperation with industry.

It is therefore important that researchers show responsibility towards the industry concerning information, so that research findings can be put into practical use.

Preprint from
CRC Handbook of Dietary Fiber in Human Nutrition
Editor Gene Spiller

BIOAVAILABILITY OF MINERALS FROM CEREALS

A review
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INTRODUCTION

Bioavailability of minerals from cereals

Cereals and cereal products are important sources of dietary fiber. Most minerals in cereals are closely associated with the outer layers, especially the aleuron cells, and have by some authors been defined as a part of the dietary fiber complex. The content of both minerals and dietary fiber increases with increasing extraction rate and unrefined cereal products have been shown to be the best single source of dietary fiber, minerals and trace elements in our diet.

However, chemical determinations of minerals and trace elements in food do not indicate the amount of the element which is available for absorption and utilization in the body. An increasing knowledge concerning the complexity of nutrient interactions and the number of dietary components that can influence bioavailability shows that this field is very complicated.

Minerals studied

Problems of practical significance concerned with the bioavailability of some minerals which are of long established nutritional importance such as calcium, zinc and iron are well known, but there seem to be similar problems for "newer" trace elements such as selenium, chromium, copper and nickel. New knowledge concerning bioavailability is constantly coming to light, and it seems that at present only a small fraction of these problems is recognized and corrected for.

Minerals and unrefined cereal products

It has been claimed that the bioavailability of minerals and trace elements in diets rich in whole grain products can be reduced in comparison with diets rich in refined cereal products. In earlier studies this was attributed to the phytic acid, present in the whole grain cereal products, and known to form complexes with divalent minerals. More recently it has been suggested that other components of the dietary fiber complex, such as fiber itself or polysaccharides might also be of importance for mineral availability. The results in the literature are conflicting and it is debatable whether it is fiber or phytic acid or both that are responsible for the decreased absorption of minerals from whole grain cereal products. In many studies bran has been added to the diet

and the effects of this on the mineral balance have been studied. It is difficult from these studies to distinguish between the effects of fiber and phytic acid on minerals, as such products have a high content of both components. It should also be realised that the addition of isolated dietary fiber components or sodium phytate to the diet may not reflect the effects of the same amount of native fiber or phytate present in the original food item, even if it may give some indication.

Since refining of cereals leads to a marked fall in the mineral content, it is important to bear in mind that even if there is an inhibition of the percentage uptake of minerals from whole grain products, this does not necessarily mean a decreased absolute uptake, due to the considerably higher amounts of minerals present.

Generalization about the bioavailability of minerals in cereals can be misleading, because the bioavailability is not the same in different cereals, due to different amounts of fiber, different fiber compositions and different amounts of phytate.

Binding behavior of minerals

The binding behavior of different minerals to cereal fibers seems to be extremely variable, probably due to the various chelating properties of the minerals. Little information on minerals other than Ca, Zn, Fe, Mg, Cu and P is available, and no agreement exists between the different minerals studied. Much of the older work is still relevant today, and has been taken into consideration when conclusions have been drawn.

Enrichment studies

When a single mineral is added to the food, as has been done in various enrichment studies, the balance between this mineral and the rest of the minerals present is changed. Studies involving enrichment of this type have also given different results from studies with the corresponding amount of the mineral naturally occurring in the food. This has been shown to be the case even with the same amounts of dietary fiber and phytic acid.

Human studies - animal studies

Much less is known about mineral requirement and bioavailability in humans than in domestic animals. Only limited studies of minerals connected with deficiency states can be carried out in humans. It is also difficult to carry out long-term studies, and studies which could cause health risks to humans would be ethically wrong. Evidence suggests that many observations carried out on animals do have relevance for man. However, direct extrapolations from animals to humans do not give the correct answers in all cases, and before any definite conclusions can be drawn, human studies are needed.

In vitro - in vivo studies

Great care is also needed when extrapolating from in vitro studies to in vivo conditions. In whole foods and meals the chelation of minerals to the different cereal components may be different than in isolated in vitro systems.

Influence of fermentation

Fermentation of the cereal e.g. during breadmaking also influences the bioavailability of minerals present in the final products. This is mainly due to the breakdown of phytic acid, and it is important to bear this in mind when conclusions are drawn concerning the final cereal product (e.g. bread) on the basis of the cereal ingredients such as flour and bran. Leavening seems to improve the utilization of minerals.

Other factors influencing bioavailability

A number of dietary factors influence the availability of minerals by either promoting or inhibiting effects. Nearly all studies done on the influence of other food items on mineral absorption concern iron, and only a few studies have been carried out on zinc absorption. Ascorbic acid seems to be the most potent enhancer of iron absorption, while animal protein improves both iron and zinc absorption. Tea on the other hand seems to depress iron absorption. When studying mineral absorption from the diet, it is important not only to study a single food component or fraction of a food component but the whole composite meal. Studies on a single food item can, on the other hand, be used to identify the dietary component's influence on the degree of absorption.

Long-term studies needed

Adaptation to a diminished availability of minerals is probably of importance after continuous fiber addition to the diet. Most of the mineral studies published so far are short-term experiments, and it is therefore difficult to draw any definite conclusions about the inhibitory/promoting factors before long-term studies have been performed.

Conclusions

There seems to be an inhibiting effect on the bioavailability of some minerals due to some, but not all, components in the dietary fiber complex in cereals. In countries where the intake of minerals is limited, and whole grain cereals account for the main part of the energy intake this could be of importance.

In normal mixed diets in industrialized countries where diets are well balanced and include animal products, the possible binding of minerals to the fiber complex is not a serious problem.

Unrefined cereal products are good sources of minerals in the human diet, and in spite of a decreased percentage absorption for some minerals, it seems that the positive balance of minerals is retained, due to the higher levels of minerals in the high-fiber products.

A lot of work is still needed in this field. The studies done up to now only give us an idea of the complexity of the bioavailability in cereals.

TABLE 1

MINERALS STUDIED WITH RESPECT TO BIOAVAILABILITY FROM CEREALS

Increased absorption	Decreased or low absorption	No change in availability
Ca 16	6, 13, 16, 17, 18, 19, 33, 48, 58, 59, 61, 66, 74, 83, 87, 88, 89, 108	4, 5, 11, 43, 44, 64, 75, 82, 84, 91, 94, 96, 99, 106, 109
P	87	4, 82, 96, 106
Zn 52, 72, 97, 98	12, 20, 34, 42, 47, 48, 65, 69, 70, 71, 73, 84, 85, 87, 88, 89, 96	4, 5, 11, 14, 31, 38, 45, 81, 82, 89, 97, 99, 103
Mn		64
Cu	21, 65	69, 97
Mg 16, 77, 106	12, 16, 48, 66, 93, 106	5, 19, 64, 79, 82, 96, 99, 110
Fe 3, 15, 39, 40, 53, 63, 95, 101	1, 2, 7, 8, 10, 12, 13, 22, 23, 24, 28, 32, 37, 46, 47, 49, 51, 54, 55, 56, 75, 78, 80, 81, 86, 88, 100, 104, 105, 107	4, 9, 16, 27, 30, 35, 36, 43, 57, 68, 82, 89, 96, 97, 99, 101, 106

TABLE 2

DESCRIPTION OF MINERAL BIOAVAILABILITY STUDIES FROM CEREALS

Mineral	Subject	Expt. design	Duration	Diet	Fiber source	Results	Ref.
Fe	Human, 8 subjects	Balance	49-91 days	Controlled 40-50 % of energy from white flour	Whole wheat meal (ex- traction rate 92 %) replaced white bread	Decreased iron abs. from brown bread, but mean balance positive	107
Fe	Rat	Hgb-repletion			Whole wheat bread	Lowered availability. Iron from bread less available than inorganic iron	80
Fe	Human, 154 subjects	Radioisotope technique	2-week period		Rice	Increased abs. with forti- fication with FeSO_4	39
Fe	Human	Hgb, serum iron IBC Radioassay ^{59}Fe , ^{55}Fe	15 days	Controlled meal	Corn	Decreased iron abs.	55
Fe, Ca, Mg	Human 3 subjects	Balance	7-day period 3-5 weeks		Brown bread (1 lb)	Fe, P, not affected. Mg, Ca abs. neg. after 4 weeks. Mg, Ca abs. pos. after 8 weeks	106
Fe	Human 66 subjects	Radioassay Food intrinsi- cally labelled with ^{55}Fe		Controlled 1 meal	Rice	Low availability	100

Abbreviations: Hgb = Hemoglobin. IBC = Iron binding capacity

(cont.)

TABLE 2 (cont.)
DESCRIPTION OF MINERAL BIOAVAILABILITY STUDIES FROM CEREALS

Mineral	Subject	Expt. design	Duration	Diet	Fiber source	Results	Ref.
Fe	Human 116 subjects	Radio-Fe, erythrocyte utilization method	14 days	Controlled 1 meal	Maize meal porridge	Very low abs. (3.8 %)	
Fe	Human 66 subjects	Radio-Fe, erythrocyte utilization method		Controlled 1 meal	Maize, wheat	Low abs.	22
Fe	Human 42 children	⁵⁹ Fe-labeled maize, whole- body counter		Controlled 1 meal	Maize	Low abs. (4.3 %)	101
Fe, Mg, Ca	Human 12 subjects	Balance	3 weeks		Unpolished rice (27 ounces)	Ca, Mg abs. lowered on un- polished rice for 3 weeks. Long-term (18 weeks): Ca, Mg, retention improved. Fe not affected	2
Fe, Zn, Cu	Human 5 subjects	Balance	28-30 days		26 g corn bran 26 g wheat bran	No significant effect with bran. Cu-abs. better because of higher intake	16 97
Fe	Human	Hgb-measure- ments			Wheat bread	Not affected	43
Fe (cont.)	Human	Balance			Different cereals	Affected by phytic acid	1

TABLE 2 (cont.)

DESCRIPTION OF MINERAL BIOAVAILABILITY STUDIES FROM CEREALS

Mineral	Subject	Expt. design	Duration	Diet	Fiber source	Results	Ref.
Fe	Human	Serum iron measurements			Whole wheat bread	Affected, possible binder phytic acid	24
Fe	Human 21 subjects	Radioisotope technique Blood samples	17 days		Chapatti from whole-meal wheat flour or white flour	Affected, but not significantly. Abs. higher from white flour than whole grain (3.2 % contra 1.7 %). Possible binder phytate	27
Fe	Human 1) 6 subj. 2) 28 subj.	Radioisotope	10 days	Self-selected	1) Rolls containing 10 % or 40 % bran 2) Rolls containing 0.3 % - 10 % bran	Decreased iron abs.	7
Fe	Human 6 subjects	Metabolic balance Serum iron measurements	3 weeks	Metabolic-controlled	36 g wheat bran	Serum iron level fell. Hemoglobin decreased	49
Fe	Rat	Hgb-repletion test	3 weeks repletion		Whole grain wheat bread	Iron utilization neg. affected. Unrelated to phytic acid or fiber	78
Fe, Ca	Human 14 subjects	Serum measurements	6 weeks	Self-controlled	20 g wheat bran	Serum iron fell, Ca unaltered	75
Fe, Zn, Ca, Mg	Human 10 subjects	Blood samples Serum mineral levels	6-12 weeks	Self-controlled	20 g wheat bran 1) Low phytate level 2) Normal phytate level	Not affected by wheat bran. Phytic acid no influence	99

(cont.)

TABLE 2 (cont.)
DESCRIPTION OF MINERAL BIOAVAILABILITY STUDIES FROM CEREALS

Mineral	Subject	Expt. design	Duration	Diet	Fiber source	Results	Ref.
Fe	Human 9 subjects	Radioassay			Bread, enriched with ferum reductum or ferric ammonium citrate	Decreased abs. with bread	
Fe	Rat	1) Chemical balance measurement 2) Radioassay	14 days	Semisyn- thetic 310 g bread per kg diet	White bread 60.5 g fiber/kg Brown bread 130.2 g fiber/kg Wholemeal bread 221.2 g fiber/kg	No difference in iron abs.	10
Fe	Human 2 subjects	Balance ex- periment Serum iron		Bread 60 % of energy	Wheat bread White bread 2 g fiber Brown bread 15 g fiber Whole grain 22 g fiber	Serum iron decreased Decreased iron balance	30
Fe					Oatmeal	Low availability. No cor- relation with phytate. Possible binder phosphorus	32 104
Fe	Human 4 subjects	Radioactivity measurements	10-15 days	Self- selected 28 g bread	White bread, exchanged for wholemeal bread	Lowered iron abs. in iron from wheat bran	28
Fe	Rat	Repletion experiment	24 days		Bread, wheat	Iron better abs. from FeSO ₄ than from bread	63
Fe	Human 11 subjects	Radioiron abs.		200 g rice (2 mg iron)	Rice	Low iron abs.	54
(cont.)							

TABLE 2 (cont.)

DESCRIPTION OF MINERAL BIOAVAILABILITY STUDIES FROM CEREALS

Mineral	Subject	Expt. design	Duration	Diet	Fiber source	Results	Ref.
Fe	Human 42 subjects	Radioiron abs.		Controlled, pancakes	Wholemeal from wheat	Geometric mean wheat iron abs. 5.1 %. 1/4 of that of FeSO ₄	46
Fe	Human 4 subjects			Self-selected meal + bread	Bran	Iron abs. 6.4 %. In one iron-deficient 19 %	9
Fe	Rat	Hemoglobin, regeneration technique			Monoferric phytate from bran	Rel. biological value of iron the same from bran and ferrous ammonium sulphate	68
Fe	Human 60 subjects	Double-isotope technique		Controlled	Whole wheat bran 12 g bran	Decreased iron abs. 51 to 71 %. Not phytic acid, but the soluble fiber fraction more than insoluble	105
Fe	Human	Isotope technique			Unmilled rice	Iron four times better abs. from milled than unmilled rice	8
Fe	Dog	Double-radio- isotope method Total-body counting			Monoferric phytate	Abs. not influenced by phytic acid	57

(cont.)

TABLE 2 (cont.)

DESCRIPTION OF MINERAL BIOAVAILABILITY STUDIES FROM CEREALS

Mineral	Subject	Expt. design	Duration	Diet	Fiber source	Results	Ref.
Fe	Human	Radioisotope technique		Controlled meal, 100 g bread	Wholemeal bread	Significantly lower iron abs. from wholemeal bread	51
Fe	Rat	Hemoglobin repletion			Corn, NDF up to 15 %	Iron 50 % less available than ferrous sulphate	37
Fe, Zn	Rat	Isotope dilution technique Fecal and urinary Zn		Semisynthetic diet, 180 g bran per kg diet	Bran	No difference in Fe-abs. due to particle size. Small effect on Zn-retention due to particle size	14
Fe	Pig	Hemoglobin repletion	7-9 weeks	Controlled Fe-intake constant	Wheat bran 20 % of diet	No difference in iron abs.	35
Mg					Bread	May be affected: cause phytate	93
Zn, Ca, Rat Mg, P		Balance experiment	41 days 2 weeks' adaptation period	Controlled	Wheat bran, 10 % and 15 %	No adverse effect on mineral abs. Favored P-retention. Adaptation	5
Zn, Cu	Human 7 subjects	Balance	32 days	Controlled	Rice, corn, wheat	Cu abs. not affected. Zn neg. affected by fiber and phytate	69

(cont.)

TABLE 2 (cont.)

DESCRIPTION OF MINERAL BIOAVAILABILITY STUDIES FROM CEREALS

Mineral	Subject	Expt. design	Duration	Diet	Fiber source	Results	Ref.
Zn	Rat	Growth response. Abs. in femur and serum	4 weeks	Controlled	Wheat bread	Not affected, probably due to completely hydrolyzed phytate during bread-making	81
Zn, Ca, P	Human	Balance, urine feces	6 days	Controlled, bread 40 % of energy. Phytate content: 680 mg and 1040 mg per day	Wholemeal bread, unleavened	Neg. effect on Ca, Zn, P	87
Zn	Pig	Growth measurements				Depressed growth due to phytic acid	70
Zn, Ca	Human	Balance, feces, plasma	20 days	Controlled 500 g bread (60 % of energy)	Wholemeal bread (25 g fiber)	Neg. Zn-balance. Neg. Ca-balance but not significant. Binder: cellulose	84
Zn	Human 4 subjects	Balance	4 weeks	Self-selected + bran	Wheat bran, 14 g	Not significantly affected by bran	38
Zn, Mg	Human 7 subjects	Balance	7 days	Controlled	Wheat bran (10-20 g) Cellulose, hemicellulose	Tendency to increased fecal Zn-loss	72

(cont.)

TABLE 2 (cont.)

DESCRIPTION OF MINERAL BIOAVAILABILITY STUDIES FROM CEREALS

Mineral	Subject	Expt. design	Duration	Diet	Fiber source	Results	Ref.
Zn	Human 28 subjects	Balance, plasma	15 weeks	Controlled Zn-intake constant	Wheatflakes, wheat bran	Neg. affected	
Ca, Zn	Human 2 subjects	Balance	20 days	Controlled metabolic ward. 50 % of energy from bread	Whole wheat bread	Neg. balance of Ca, Mg, Zn, P	42
Zn	Human 66 subjects	Radioisotope technique. Whole-body counter		Controlled	Whole grain bread	Decreased percentage abs. but increased total abs. Possible binder: phytic acid	85
Zn	Chicken	Growth response	4 weeks	Controlled	Corn	Decreased abs. Only 60 % zinc utilized, possible binder: phytic acid	98
Zn	Rat	Femur zinc measurement		Controlled	Rice, barley	Barley poorer source than mixed infant cereal (wheat, corn, oat). Rice poorer than standard. No correlation between Zn-availability and fiber/phytate content	71 103

(cont.)

TABLE 2 (cont.)

DESCRIPTION OF MINERAL BIOAVAILABILITY STUDIES FROM CEREALS

Mineral	Subject	Expt. design	Duration	Diet	Fiber source	Results	Ref.
Zn	Rat	Repletion experiment and growth response	28 days		Wheat bran 50 g fiber/kg	Reduced growth. Decreased zinc abs. (phytate possible binder)	20
Zn	Rat	Weight gain, femur zinc content			Iranian flat bread, bread and dough	Bread better Zn source than unfermented dough. No difference in dough with different fermenting times. No correlation between Zn and fiber/phytate	31
Zn	Rat	Growth response			Selected cereals	Phytic acid inversely related to zinc availability. Ratio Zn: phytate important for Zn-availability. More inhibition of phytic acid in cereals than legumes	34
Zn	Rat				Grain	Availability of Zn in grain better than in legumes. Phytic acid not the only factor responsible for decreased availability	52
Zn	Rat	Balance experiment and ^{65}Zn kinetic			Cereal	Phytic acid naturally occurring in food, affects Zn metabolism to the same extent as Na phytate	45

(cont.)

TABLE 2 (cont.)

DESCRIPTION OF MINERAL BIOAVAILABILITY STUDIES FROM CEREALS

Mineral	Subject	Expt. design	Duration	Diet	Fiber source	Results	Ref.
Zn	Rat	Balance Growth re- sponse		Controlled	Breakfast cereals: rice, corn	Depressed growth when molar ratio > 15. Biological re- sponse not directly corre- lated to dietary fiber in cereals. Phytate: Zn ratio major factor affecting Zn- availability	67
Zn, Ca	Rat	Metabolic balance			Wheat bran, 0.5 %, 10 %, 15 %	No significant changes	4
Zn	Rat	Balance studies			Rye	Apparent Zn-abs. and re- tention in absolute values, higher from flour with high extraction rate	73
Ca, Mg, Na, K, Cl	Human 6 subjects	Balance exper- iment	3 weeks	Controlled	Different cereals. Fiber intake increased from 17-45 mg/day (Bran, wholemeal)	Decreased Ca-absorption. K, Na, Cl small changes. Fecal Mg increased, but probably due to higher Mg-intake	19
Ca	Human	Balance studies	4-9 weeks		Wholemeal bread	No change or adaptation	106
Zn, Ca, Mg	Human 2 subjects	Balance	5-6 weeks		Wholemeal bread	Neg. affect on Ca-abs. Ca-, Zn-adaptation	11
Ca (cont.)	Human 19 subjects	Serum measurements	19 weeks	Controlled 230 g bread	Wholemeal bread	Not affected	43

TABLE 2 (cont.)

DESCRIPTION OF MINERAL BIOAVAILABILITY STUDIES FROM CEREALS

Mineral	Subject	Expt. design	Duration	Diet	Fiber source	Results	Ref.
Ca	Human 27 subjects	Balance	6 weeks		Bran (10-20 g)	Lowered Ca in serum	74
Ca	Human	Balance			Wheat wholemeal bread, unleavened	Decreased Ca-abs. Possible binder: phytic acid	6
Ca	Human	Balance			Wholemeal bread, chapatti	Decreased Ca-abs.	83
Ca	Human	Balance	7 weeks		Wholemeal bread	Decreased Ca-abs.	33
Ca	Human	Balance	3 weeks		Wholemeal bread, bran (22 g - 53 g fiber)	Neg. Ca-balance even if increased Ca-intake	18
Ca	Human				Whole grain	Decreased Ca-abs.	108
Ca	Human 9 subjects	Balance	9 months, Periods: 2-3 weeks	Flour 40- 50 % of energy	Whole grain flour (extraction rate 92 %)	Neg. Ca-balance. Possible binder: phytate. Dephyt- inization gives better abs.	59
Zn, P, Ca,	Human 3 subjects	Balance	28+32 days	Controlled Bread 350 g	Whole grain bread, un- leavened or leavened	Unleavened bread: Neg. Ca- and Zn-balance. High phytate intake can cause neg. disturbance in Zn- and Ca-adaptation for serum-Fe and serum-Zn	89

(cont.)

TABLE 2 (cont.)

DESCRIPTION OF MINERAL BIOAVAILABILITY STUDIES FROM CEREALS

Mineral	Subject	Expt. design	Duration	Diet	Fiber source	Results	Ref.
Ca	Puppies	Balance			Different cereals	Different cereals had different effects on Ca. Oat inhibited Zn-abs. most, and more than wheat, rye, barley. Possible binder: phytate, phosphate as such not responsible	61
Ca	Human 4 subjects	Balance	3 weeks		Wheat fiber, 31 g fiber	Neg. Ca-balance	17
Ca	Human 6 subjects	Balance	11 days	Controlled	Wholemeal wheat bread fiber 123 g (standard 106 g)	Neg. Ca-balance	58
Ca	Human 14 subjects	Balance (serum)	4-9 weeks	Controlled	Wheat bran, 38 g bran/day	No changes in plasma-calcium	44
Ca	Human 25 subjects	Balance (serum)	5 weeks	Controlled	Wheat bran 24 g bran/day	No changes in serum-calcium	109
Ca	Survey children	Balance	1940 - 1948	Self-selected	Wholemeal bread	Ca-adaptation. No warning for Ca-abs.	94
Ca, Mg Zn, P	Human 2 subjects	Balance		Controlled 60 % of energy from bread	Wholemeal bread Fiber 12.6 g/day	Neg. Zn-balance. Neg. Ca-balance. Neg. Mg-balance	48

(cont.)

TABLE 2 (cont.)

DESCRIPTION OF MINERAL BIOAVAILABILITY STUDIES FROM CEREALS

Mineral	Subject	Expt. design	Duration	Diet	Fiber source	Results	Ref.
Zn, Cu, Fe, Mg	Rat	Balance			Phytic acid	Decreased abs.	21
Mg	Rat	Balance		Wheat flour, 55 % Mg, the rest of Mg from magnesium-carbonate	Whole grain wheat bread	Mg available	
Mg	Rat	Balance	3 weeks		Whole grain wheat bread	Mg available	110
Mg	Rat	Balance	4 weeks		Whole grain wheat flour	Mg available. Higher abs. from flour than from the same amount added Mg-salt	79
Ca, P, Zn, Mg	Human 68 subjects		13 months	Self-selected	3 tsp. bran/day	No influence on mineral balance. Bran does not cause deficiency	77
P, Ca, Mg, Zn, Fe	Human 8 subjects (ileostomy)	Metabolic balance technique	2-3 weeks	Controlled	Wheat bran, 16 g/day	No effect on mineral abs. except Zn	82
							96

TABLE 3
IN VITRO BINDING STUDIES

Mineral	Fiber source	Results	Ref.
Ca, Mg, Zn, Fe	Wheat bran and fractions of dietary fiber	Lignin, pectin high metal-binding capacity. Metal-binding pH-dependent.	13
Fe, Cu, Zn	Rice bran	All three metals bound, probably to hemicellulose. Sequence of binding: Cu > Zn > Fe. Metals released with enzyme incubation. Zn affected dietary Cu-binding.	65
Zn, Fe	Wholemeal wheat bread	Bran high binding capacity for Zn and Fe. Zn-binding pH-dependent. Lignin and two fractions of hemicellulose: high capacity for Zn. Complexes with wheat fiber can, to some extent, explain decreased availability of Fe and Zn.	47
Fe	Wheat, rice	Ionisable iron from bread nearly twice as high when compared with that from chapatti prepared from same wheat. Percentage ionisable iron was lower in the parboiled rice than in raw rice, but actual amount the same. Cereal, twofold increase of ionisable iron when germinating. Cooking of rice or wheat did not influence ionisable iron.	76
Fe	Wheat, maize	Neutral detergent fiber accounted for all binding capacity of iron. Binding pH-dependent. Iron binding by fiber is strongly inhibited by ascorbic acid, citric acid, phytic acid, EDTA, cysteine, phosphate and calcium. Iron in wheat and maize strongly inhibited.	86
Fe	Wheat bran	Ascorbic acid inhibits binding of ferrous iron to wheat bran. Boiling for 1 hr - no effect on phytic acid. Toasting for 1 hr - 19 % destruction of phytic acid. Boiling for 1 hr in 1 N HCl - 36 % destruction of phytic acid.	12

(cont.)

TABLE 3 (cont.)
IN VITRO BINDING STUDIES

Mineral	Fiber source	Results	Ref.
Zn	Rye bread and whole wheat bread	Higher availability with increased rising time.	41
Zn	Whole wheat bread	Dephytinized fiber did bind Zn. Adding of phytic acid lowered the ability to bind Zn.	84
Zn	Bran, and components of bran	Soluble components responsible for 39 % of total binding power of wheat bran. Possible binder: phytate. Hemicellulose, cellulose, starch & pectin contribute only 10 % of total binding capacity.	32
Zn, Fe, Ca, Mg	Wheat bran	Zn, Fe, the only soluble metals of wheat bran associated with phytic acid. At least 70 % of phytate does not exist as Ca ₅ Mg-phytate.	26
Ca	Wheat bran	Only weak binding. No important contribution to Ca-binding. Increased binding with lowered pH (pH under 5).	91
Ca, Mg	Cell wall fraction	Cellulose, hemicellulose: low availability.	66
Mg, Mn, Ca	Rice bran	Minerals available for absorption. Minerals released after incubation for 16 h.	64
Fe	Phytate, Oatmeal	No correlation with phytate and Fe-absorption. Oatmeal low availability, phosphate?	104
Ca, Zn, Fe	Bread	Cellulose important binder. Fiber responsible more than phytic acid.	88

(cont.)

TABLE 3 (cont.)
IN VITRO BINDING STUDIES

Mineral	Fiber source	Results	Ref.
Ca, Mg, Zn, Fe	Wheat bran	Binding to cellulose and lignin not affected by cooking. pH-dependent Ca-binding diminished by boiling, not affected by toasting. Zn affected significantly by pH and type of cooking. Toasting had no effect. Boiling no effect at pH 6, decreased binding after boiling at pH 7.	13
Zn, Cu, Fe	Corn	Cornflakes bound more Cu, and less Fe than corngrits at pH 5, 6 & 7.	50
Na, K, Ca, Mg, P	Wheat bran	Ash associated with soluble fiber, coprecipitation due to isolation procedure of fiber partly responsible. Dependent on ionic strength and different buffers.	102
Ash, general	Different cereals	Ash associated with soluble fiber components.	36
Zn	Wheat bran	Soluble comp. responsible for 37 % of total binding power of wheat bran. Possible binder phytate. Hemicellulose, cellulose, starch, pectin contribute only 10 % of total binding capacity.	92
Fe	Wheat bread	Insoluble iron generated due to baking process. The differences found between iron sources prior to baking vanished in final baked product.	56

TABLE 4

EFFECT OF ADDITION OF OTHER FOODS ON BIOAVAILABILITY OF MINERALS

Mineral	Fiber source	Subject	Food addition	Results	Ref.
Fe	Rice	Human	Fish	Increased abs.	39
Fe	Corn, maize Bread	Human	Vitamin C	Increased abs.	15
Fe	Rice	Human	Fruit, meat	Abs. increased 5 times	40
Fe	Maize	Human	50 g meat 100 g fish 150 g papaya (66 mg vit C)	Abs. doubled with meat and fish. Increased abs. 5 times with papaya	53
Fe	Corn	Human	Fish or food of animal origin	Increased availability	55
Fe	Bread	Human	Tea	Decreased availability	23
Fe	Rice	Human	Ascorbic acid	Improved availability.	100
Fe	Maize	Human	Tea Ascorbic acid	Tea reduced abs. from 3.8 % - 2.1 %. Ascorbic acid increased 10 times. Inhibitory effect of tea overcome if ascorbic acid given	22
Fe	Maize, wheat	Human	Ascorbic acid	Enhanced abs. from maize, not from wheat	101
Fe	Cereals	Human	Ascorbic acid	Improved <u>non heme</u> iron abs.	29
Zn	Whole grain bread	Human	Animal protein Milk (Ca)	Both improved abs.	71
(cont.)					

TABLE 4 (cont.)
EFFECT OF ADDITION OF OTHER FOODS ON BIOAVAILABILITY OF MINERALS

Mineral	Fiber source	Subject	Food addition	Results	Ref.
Fe	Whole grain bread	Human	Tea Orange juice Eggyolk	Tea reduced abs. to half Orange juice increased abs. by factor 2 Eggyolk inhibitor	71 95
Fe	Wholemeal bread	Human	Fruit juice Egg	Fruit juice enhanced abs. Egg no effect	28
Fe	Rice	Human	Fish	Abs. improved with fish	3
Fe	Wheat bread	Human	Orange juice	Improved abs.	10
Fe	Wholemeal bread	Human	Ascorbic acid	Enhanced abs.	51
Zn	Flat bread	Rat	Protein (animal)	Could prevent complexation of Zn with phytic acid	31

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AIMS OF THE STUDY

The aims of the present study were:

1. To measure the content and variation of dietary fiber in different flours and cereal products on the market in Norway.
2. To get an idea of the content and variation of whole grain flour in Kneipp bread, which is the most frequently consumed bread in Norway, and to get an impression of how the acceptability of this bread varied with the content of whole grain flour.
3. To investigate in vitro the complexation of minerals to the different components in the dietary fiber complex, including phytic acid, and how this is influenced by the baking procedure.
4. To study in vivo the bioavailability of minerals from whole grain cereal products in long-term studies in animals and man.

TERMINOLOGY AND DEFINITION OF DIETARY FIBER

In 1972 Trowell proposed a definition of dietary fiber as "the remnants of the plant cell wall that are not hydrolyzed by the alimentary enzymes of man". In 1976, the definition was expanded, to include the "plant polysaccharides and lignin which are resistant to hydrolysis by the digestive enzymes of man". In this dietary fiber concept, storage polysaccharides, gums, mucilages, algal polysaccharides as well as food additives of this nature are included.

The polysaccharides include cellulose which is a pure glucose polymer and hemicellulose, pectins, mucilages, gums and other polysaccharides which have quite variable monomeric compositions. Lignin is a complex polymer based on phenyl propane units.

Dietary fiber is associated with components which are not of a fibrous nature. These undigestible substances, such as minerals, proteins, waxes, cutins and other cell-wall-bound substances, have, together with dietary fiber been called the "dietary fiber complex" (Spiller, 1975; Trowell, 1976).

In food, the dietary fiber components do not exist as well-defined single molecules, but as large aggregates together with the other undigestible components.

Vitamins and minerals are usually concentrated to the fiber-rich parts of the plant. During the refinement of food, not only the dietary fiber components, but also the mineral and vitamin levels will diminish.

Some authors (D.A.T. Southgate, 1982) have claimed that the dietary fiber concept should be related only to natural food items, and that dietary fiber could then be regarded as a measure of the "degree of unrefinement" of the food.

DETERMINATION OF DIETARY FIBER

The definition and methodology of dietary fiber are closely related.

There is, in the dietary fiber components, no common characteristic element or chemical group which can be used as an indicator for dietary fiber, and due to the variability in composition and structure, the analysis of dietary fiber has been the subject of much discussion, and agreement on a common method has not yet been reached.

Methods for quantitative analysis can be divided into two groups:

1. Fractionation methods, where the dietary fiber monomers are assayed specifically.
2. Gravimetric methods, which include removal of non-fiber components from the sample, before weighing of the dietary fiber.

Fractionation methods

Fractionation methods using colorimetric or gas chromatographic techniques give important detailed information and are necessary when determining the specific dietary fiber components. Many methods determine the neutral monomeric components obtained by separation and hydrolysis of the dietary fiber components, using gas chromatographic separation and quantification.

Uronic acids are determined separately (Theander and Aman 1979, 1982; Selvedran et al, 1979; Englyst et al, 1982).

In Southgate's colorimetric method (Southgate, 1969, 1976) the residue obtained after enzymatic removal of starch, is separated into cellulose, non-cellulose polysaccharides and lignin. The polysaccharides are then determined colorimetrically. Lignin is, in most cases, determined as Klason lignin, i.e. the residue insoluble in 72 % H_2SO_4 . More specific methods have been developed by Robertson and Van Soest (1977).

Gravimetric methods

Crude fiber was, for a long time, the only accepted method for measuring the fiber content in foods. This method measures only a small and variable amount of the total dietary fiber. No standard factor can be used to convert crude fiber values to dietary fiber values.

Detergent methods were developed by Van Soest and coworkers (1977), originally for forage fiber, but the use of it in human foodstuffs resulted in problems. The residue obtained with the neutral detergent method (NDF) consists mainly of hemicellulose, cellulose and lignin, and is significantly contaminated by starch and/or protein.

Modifications of the method including amylase digestion have been proposed for starchy materials (Robertson and Van Soest, 1977; Schaller, 1977).

The acid detergent fiber method (ADF) includes cellulose, lignin and some other substances (cutin, silica).

Hemicellulose is dissolved in the acid detergent solution, and the difference between NDF and ADF has been used to estimate the hemicellulose content. The ADF method, however, has been shown not to be absolutely specific for cellulose and lignin, and the residue has been reported to contain pectin (Belo and DeLumen, 1981).

Enzymatic methods are based upon hydrolysis of protein and starch by enzymes. Most of the methods measure only insoluble dietary fiber components, separated from the enzyme digestion by centrifugation and/or filtration (Weinstock et al, 1951; Hellendoorn et al, 1975; Elchazly and Thomas, 1976). A modification in which soluble components are recovered by alcohol precipitation and another filtration/centrifugation have been published (Schweizer et al, 1979; Furda 1981; Asp et al, 1983). Detergent methods cannot be developed in this way, since the soluble polysaccharides cannot be easily separated from the detergent-soluble protein and starch.

When choosing enzymes it is important that the enzyme preparations are not contaminated with dietary-fiber-splitting enzymes, e.g. hemicellulases or pectinases.

The previously used enzymatic methods employed long incubation times (20 - 30 hours) and often included laborious centrifugation procedures to recover the dietary fiber fractions.

Asp and coworkers (1983) developed their method further to overcome these problems. The incubation time has been shortened to 2 x 1 hour without much loss of efficiency. Starch removal has also been improved by including a heat-stable enzyme, Termamyl, in the gelatination step, which has resulted in practically no starch being detected in the residue even with very starchy materials.

In the method of Asp and coworkers both insoluble and soluble dietary fiber are recovered by filtration.

A method suggested to the AOAC (Association of Official Analytical Chemists) for total dietary fiber (Prosky et al, 1984) is based on that of Asp and coworkers (1983) but with other enzymes. These two methods give practically the same results and are in good agreement with detailed methods (Prosky et al, 1984; Varo et al, 1983).

Corrections for undigestible protein and ash associated with the fiber components are necessary to make the enzymatic gravimetric assay accordant with the definition of dietary fiber.

The choice of method to be used for dietary fiber analysis depends upon the purpose of the analysis. Detailed analysis is necessary for many scientific investigations. But in addition to these informative, but also more laborious methods, there is a need for simple and rapid methods, not only for research purposes, but also for food labeling and control. In these cases there is no need for a detailed characterization of the fiber components to obtain the total fiber content, in the same way that there is no need for an analysis of all the amino-acids to give the protein content.

In the present study, no detailed information about fiber monomers was needed, and the enzymatic gravimetric method of Asp and coworkers was chosen. This method has a high reproducibility for cereals as well as for fruit, legumes and vegetables. It is a comparatively rapid method, giving the content of both insoluble and soluble fiber components. As the method of Asp and coworkers is a non-destructive method, the residues obtained of the two fractions by filtration or centrifugation can be used in further investigations.

RESULTS AND DISCUSSION

DIETARY FIBER IN FLOURS AND CEREAL PRODUCTS IN NORWAY

In paper I, the dietary fiber content and variation of whole grain rye flour and four different milling fractions of wheat were determined from the nine different mills in Norway.

Considerable variation was found in the whole grain flours, whereas the fiber content of brans was found to be almost constant.

The total dietary fiber in whole grain rye varied from 13.9 % to 23.3 %, in whole grain wheat from 11.9 % to 17.9 % and in white wheat flour from 2.8 % to 4.6 %. The figures are all given on a dry matter basis. The variation in bran I (including both outer layers and germ) was from 51.5 % to 52.9 %, and in bran II (almost only outer layers) from 57.2 % to 62.2 %. Due to the high dietary fiber content in the brans, the relative variation was smaller than for other milling fractions.

The variation in the dietary fiber content in the whole grain flours may well be explained by the substantial import of cereals for human consumption into Norway. The origin of the grains will vary and consequently the mixture of grains at the different mills will also vary. For food labeling, therefore, using an average value from all the mills for each type of flour will probably be the best solution. The variation must be taken into consideration when it comes to food labeling and control of cereal products.

The variation in the dietary fiber content in the white flours also reflects the variation in the extraction rate. The extraction rate in Norway is defined to be 80 %, which should be adjusted according to the ash content. This is, however, too time-consuming in the mill's daily routine, and in practice the extraction rate is determined by the grade of the color of the white flours. As there are differences in the color of flours from different origins, this will result in a wide variation in the ash content. The 80 % extraction rate is therefore only to be looked upon as an average figure (personal communication K.M. Fjell, 1984). This is one explanation for the differences found in the ash content of white flours in paper I.

From our data we could not establish a correlation between the ash content and the dietary fiber content in the white flours.

Dietary fiber is probably a better measure of the extraction rate in some cereals. In wheat, however, the dietary fiber content is relatively constant when the extraction rate is under 80 %. This means that for wheat flours with a low extraction rate, dietary fiber is not a good measure either (Nyman et al, 1984).

In processed products with an unknown addition of salt, or flours which have been enriched with minerals, ash content is not a good measure of the content of whole grain flour. Also, for these products, the dietary fiber content would be a more suitable measure.

In conclusion the dietary fiber content is probably the most suitable parameter for estimating flours with high extraction rates (wheat flours over 80 %) and for determining the content of whole grain flour in processed products. One should, however, be aware of the possibility of isolated fiber components being added to the products.

In paper II, the dietary fiber content of other cereals and cereal products on the market in Norway was determined.

Barley (extraction rate 50 - 70 %) and oatflakes had almost the same content of dietary fiber as whole grain wheat and rye, 15.2 % and 11.7 % respectively. When determining the insoluble and soluble dietary fiber fractions in the four different grains, it was found that the proportions of these two fractions were different. In whole grain wheat and rye, the soluble fiber fraction accounted for 10 - 20 %, while in barley and oats up to 50 % of the total dietary fiber was comprised of soluble fiber components.

Knowledge about the correlation of insoluble and soluble fiber components is valuable when determining the content of whole grain flour from dietary fiber content. The addition of fiber, which is not of cereal origin, to bread could, in this way, be revealed, as the correlation between the fiber components would most probably be altered.

For both wheat and rye, the content of soluble fiber seems to be independent of the extraction rate (paper I; Nyman et al, 1984).

Cereal products in Norway have a very variable dietary fiber content. Even if they are called "high in fiber" on the package, the content is sometimes under 10 %. There are, however, products on the market with high dietary fiber content for consumers requiring these products. Labeling of the products,

however, is necessary for the consumer when choosing food products with a high dietary fiber content.

Paper III, reports on a study of the content of whole grain flour in bread. By means of the dietary fiber estimation, the content and variation of whole grain flour in the most commonly used bread in Norway (Kneipp bread) was investigated.

A linear correlation between the content of dietary fiber and the content of whole grain flour in bread has been shown (Johansson et al, 1984).

Heat treatment of cereal products, however, is known to increase the amount of total dietary fiber through formation of resistant starch (Johansson et al, 1984) and "lignin" formation due to the Maillard reaction (Theander et al, 1983). In bread most of the increment is due to the formation of resistant starch (Johansson et al, 1984).

When converting the dietary fiber content into the content of whole grain flour in processed products, the standard curve used for this purpose must be based on products which have passed through the same heat procedure as the product studied.

In paper III it was found that the dietary fiber content in bread was higher than expected from the analysis of flours. This was also the case in another study (Hoff and Frølich, 1984, unpublished results), where the difference between the content of dietary fiber from the analysis of bread and the dietary fiber content calculated from the content of fiber in flour could be as much as 25 %.

Therefore, in the work described in paper III the content of dietary fiber was estimated in the bread samples and the whole grain flour content was calculated from a standard curve based upon bread.

In the bread samples collected during 1983, a variation in the dietary fiber content from 3.9 % to 7.6 % was observed. This corresponds to a variation of 20 % to 65 % in the whole grain flour content. 80 % of the bread had more than 35 % whole grain flour, with a content of 45 % being most frequent.

In a study from 1979 - 1980, a content of 30 % whole grain flour was most frequent in this type of bread, the Kneipp bread.

It should, however, be mentioned that this whole grain flour content has been calculated from the recipes of 70 bakeries.

But even if the content of the whole grain flour is calculated in two different ways, these two studies show that there is a clear tendency towards a higher content of whole grain flour in Kneipp bread. The explanation for this increase over 3 - 4 years could, for the main part, be the efforts of researchers and industry in giving information to the consumer and trying to increase the content of whole grain flour in cereal products.

Another factor of importance for this increase could also be the improvement by the Norwegian Grain Cooperation of whole grain flour in Norway, with an increased protein content, making it possible to make a bread with a higher whole grain flour content (Fjell, 1980; Frølich, 1981).

Sensory analysis of bread samples was carried out, measuring properties that have previously been shown to be of importance in Kneipp bread. The scores were mainly near the middle of the scale used, which indicates that the products had no very strong characteristics. When correlating the sensory properties to the content of dietary fiber, there turned out to be some positive correlations.

The total impression regarding the crumb and crust, flavor, texture of the crust and salty taste were all positively correlated to the dietary fiber content. This means that a high content of whole grain flour gives a high score for typical bread flavor and total impression of the bread, and contributes positively to the sensory properties of the bread.

IN VITRO STUDIES OF MINERAL BINDING

Furda (1979) has indicated that calcium ions are bound to pectin and hemicellulose molecules by electrostatic attractive forces.

McHale et al (1979) have claimed that calcium and magnesium absorption could be inhibited by hemicellulose and cellulose present in the diet. Dietary phytate has been shown to reduce the availability of zinc, iron, calcium and magnesium (Oberleas 1973). Reinhold et al (1975) measured the binding of zinc, iron and calcium to bread, flour and bran, both chemically and enzymatically dephytinized, and concluded that fiber rather than phytate was responsible for the complexation of divalent cations. This is in accordance with the study of Simpson et al (1981) showing that the inhibitory effect on iron in bran is not attributable to the phytate content, but rather to dietary fiber components.

Davies et al (1977) and Nävert et al (1983) showed that the phytate content in bran and whole grain bread could, on the other hand, account for almost all the reduced zinc availability, whereas fiber components were without significant effect.

Thus, data is conflicting and no agreement exists on the relative importance of fiber and phytate for the binding of minerals to the dietary fiber complex.

In paper I in the present study the dietary fiber complex and especially the soluble fiber components from cereal and cereal products were associated with considerable amounts of ash, when assayed with an enzymatic, gravimetric method, using ethanol to recover soluble fiber components. Only minor amounts of ash could be detected in the insoluble fiber fraction.

In papers IV, V and VI we tried to find an explanation for this observation and to study how the breakdown of phytic acid during fermentation of bread or through phytase addition influenced the mineral binding in vitro. One must, however, be very careful when predicting in vivo effects on the bioavailability from the in vitro binding studies.

In the work described in paper IV we investigated different modifications of the digestion and isolation procedure on mineral association to the fiber components. Wheat bran was chosen as a fiber-rich material, because it is also rich in minerals and phytic acid, which makes it easy to follow these three parameters.

Buffer choice, ionic strength in the solution and the fiber isolation procedure turned out to be important.

By choosing a buffer with a low ionic strength, substituting citrate for phosphate buffer and isolating soluble fiber components by dialysis instead of precipitation with ethanol, the ash in the fiber fractions was considerably reduced. However, the minerals could never be completely dialysed, indicating that minerals were complexed to the fiber fractions, mainly to the soluble fraction. When using citrate buffer, it should be pointed out that there was a substantial increase in the residual protein in the soluble fiber components. Therefore, before recommending the alternative use of citrate buffer in the assay of dietary fiber components, more efficient proteolytic steps have to be introduced into the procedure.

A close relation between the phytic acid content and the ash content associated with soluble fiber components was observed, even in cereals with widely differing phytic acid contents.

In papers V and VI a bread dough with a high whole grain flour content and bran was chosen as a fiber source, in order to investigate the effect of the baking procedure on dietary fiber, phytic acid and the binding of some selected specific minerals.

In paper V we showed that the isolation procedure of the fiber components was important for their mineral binding capacity. The different cations investigated were not uniformly affected by the isolation procedure. Coprecipitation of the minerals present in the buffer and enzymes in the method of Asp and co-workers (1983) gave high amounts of phosphorus and amounts of calcium and zinc exceeding those in the dough samples. For this reason citrate buffer was used in mineral studies and the enzyme Termamyl was omitted, to avoid unnecessary mineral addition.

The purification procedure for the fiber components seems to cause chemical and physical alterations in the fiber complex, which may influence its behavior in mineral complexation. Great care must therefore be taken when extrapolating from studies on isolated fiber components to the original fiber source. This has also been pointed out by other authors (Fernandez et al, 1982; Leigh et al, 1983; Story et al, 1983).

Phytate in the dietary fiber complex

Phytate (myo-inositol-hexaphosphate) occurs naturally in many foods derived from plants (figure 1). It is the storage form of phosphorus and other minerals in seeds and cereal grains. 60 - 90 % of the total amount of phosphorus in plants is found as phytic acid (Asada et al, 1969; Reddy et al, 1982). Most of the phytic acid is located in the aleurone cell layer and with increasing extraction rate, the content of phytic acid will increase.

In our investigations (paper II) 70 % of the total phosphorus was present as phytic acid, which could give an explanation for the close relation between phytic acid and ash in the soluble fiber fraction found in paper IV, as up to three quarters of the phytic acid was detectable in the soluble fiber fraction. No phytate, on the other hand, could be detected in the insoluble fraction. The rest of the phytate was recovered in the supernatant after precipitation and filtration of the dietary fiber components.

During the baking procedure (paper VI) the phytate content was gradually reduced, and after 17 hours of fermentation it was about half of that present in the original dough at zero time. While the phytate content in the supernatant after alcohol precipitation seemed to be constant during fermentation, the phytate associated to the soluble fiber fraction was broken down to the same extent as the total phytate in the dough. This result was found both when the soluble fiber fraction was recovered by dialysis and by precipitation/filtration.

Thus the main part of the phytate is recovered together with the soluble fiber components. This could be explained by an association to the soluble fiber components or by the phytate molecules forming large aggregates. In both cases the phytate will be coprecipitated with ethanol (together with the soluble fiber components), and also, because of its size, be hindered from passing the dialysis sack (exclusion limit 6,000 - 8,000 Dalton).

A small part of the phytate seems neither to be associated to soluble fiber components nor to other phytate molecules and is small enough to pass the dialysis sack and will not be coprecipitated with ethanol.

After an additional 18 hours of hydrolysis of the soluble fraction with added phytase, prepared from wheat, the phytate was broken down so that only 5 -

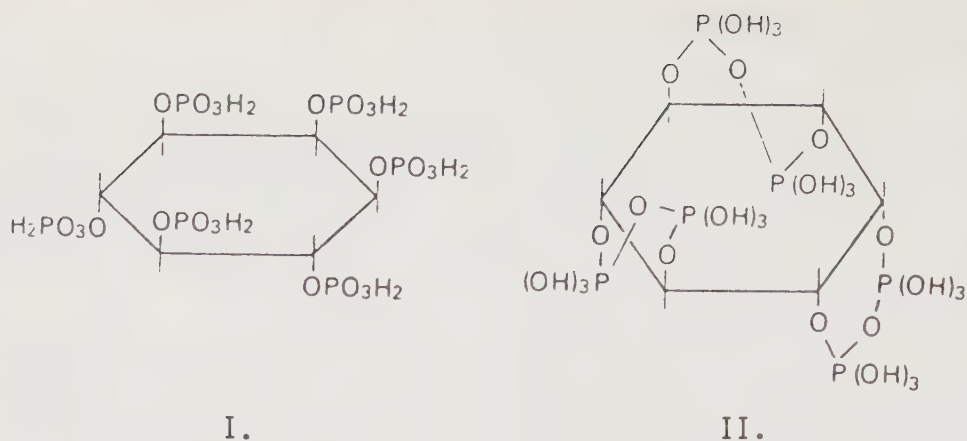


FIGURE 1.1.

Proposed structures of myo-inositol hexaphosphate

I. Anderson, R.J., 1914

II. Neuberg, C., 1908

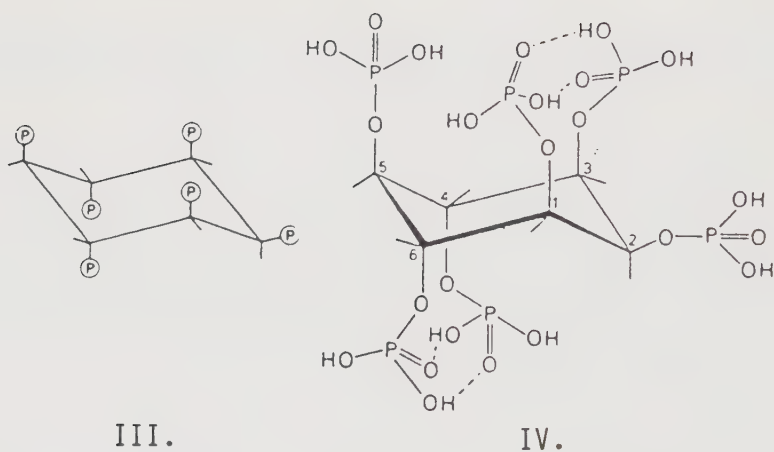


FIGURE 1.2.

Proposed conformation of myo-inositol hexaphosphate

III. Blank, G.E. et al 1971

IV. Barré, R. et al 1954

6 % of the original phytate could be recovered. In our experiments a complete hydrolysis of phytate was never obtained.

The fate of phytate during hydrolysis was also followed by using ^{31}P -NMR spectroscopy in an isolated system (W. Frølich and T. Drakenberg 1984, unpublished results).

When using this method, pure myo-inositol-hexaphosphate (phytate) resulted in a ^{31}P -NMR spectrum with four resonances with the intensity ratio 1:2:2:1 (figure 2), which is in agreement with the symmetry of the molecules (Johnson et al, 1969; Isbrandt et al, 1980).

The figure shows the time dependence in the ^{31}P -NMR spectrum for a solution containing 8 mg phytate/ml and 1 ml of a prepared phytase solution (paper VI).

From the figure it seems likely that the phytase attacks in a step-wise manner. The breakdown thus seems to proceed in such a way that the inositol-pentaphosphates formed after hydrolysis of inositol-hexaphosphate will not be hydrolyzed further, or only to a small extent, before all the inositol-hexaphosphate is broken down. The inositol-tetraphosphates are observed only when practically all the inositol-hexaphosphate has disappeared. After 5 to 6 hours, only tri-, di-, and monophosphate of the inositol could be detected. The effects of tetra- and triphosphates on the bioavailability of nutrients are not known. These inositol-phosphates have not been found to occur in mature plant seeds. During fermentation, however, these inositol-phosphates are likely to occur, and have been found by Tomlinson et al (1962).

Tangkongchitr et al (1981) have observed the step-wise breakdown of phytate, and Mihailovic et al (1965) have observed it during the germination of grain.

Dietary fiber

During fermentation and baking the total dietary fiber content was not influenced. A slight tendency, however, towards an increased amount of insoluble fiber components during baking could be observed, probably due to the formation of resistant starch (Johansson et al, 1984).

Minerals in the dietary fiber complex

Determination of individual selected minerals generally showed a similar distribution between insoluble and soluble fiber fractions as the total ash.

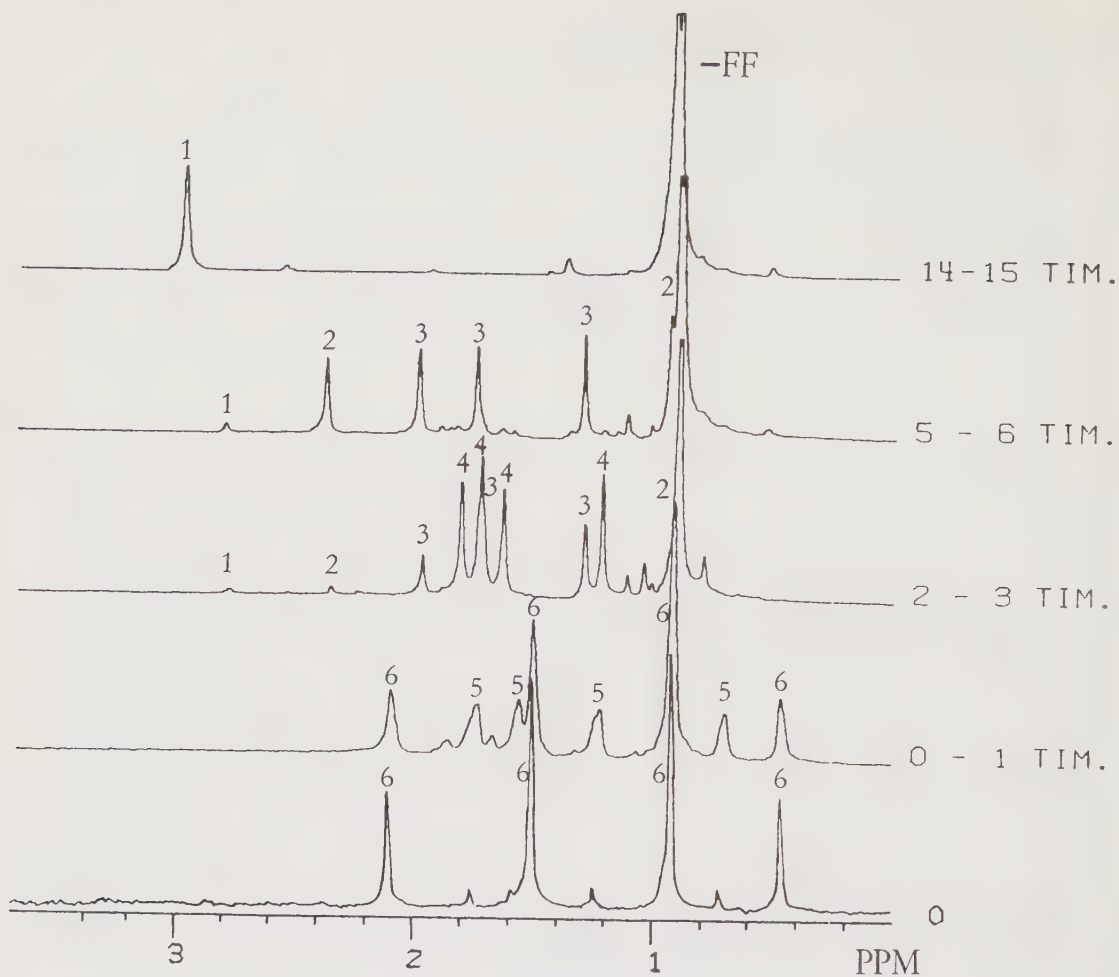


FIGURE 2.

Hydrolysis of pure myo-inositol hexaphosphate (8mg/ml) with phytase prepared from wheat (4mg/ml) in an isolated system using ^{31}P NMR-spectroscopy at pH 5.1 at 24°C at different time intervals.

Peaks marked 6- resonance from inositol hexaphosphate

Peaks marked 5- resonance from inositol pentaphosphate

Peaks marked 4- resonance from inositol tetraphosphate

Peaks marked 3- resonance from inositol triphosphate

Peaks marked 2- resonance from inositol diphosphate

Peaks marked 1- resonance from inositol monophosphate

Peaks marked FF- resonance from free phosphate

Different minerals, however, behaved differently both concerning binding to fiber fractions before fermentation and behavior during fermentation.

The amount of ash associated with insoluble components was small and not influenced by fermentation. When comparing with the minerals present in the original dough, 35 - 40 % of the iron, however, was present in this fraction. 23 % of the calcium and 16 % of the zinc, but only 3 - 4 % of the magnesium and phosphorus present in the original dough were present in the insoluble fraction.

The picture for minerals associated to the soluble fiber components was different. As percentage of the mineral present in the original bread dough, 61 % of the calcium, 29 % of the iron and 24 % of the zinc and phosphorus (but only 9 % of the magnesium) were recovered in this fraction.

During fermentation there was a decrease in the minerals associated to the soluble fiber fraction. Zinc, magnesium and calcium seemed to be associated to phytic acid, as their release during fermentation ran parallel with the phytate hydrolysis.

However, only 10 % of the total iron present in the bread dough was released during fermentation, 60 % of the iron was still not dialyzable after nearly complete phytate breakdown and thus seemed to be associated to fiber components, both insoluble and soluble, not being affected by fermentation. This is in accordance with the studies of Verma et al (1977) and Schricker et al (1982) showing no significant changes in the iron availability during the baking procedure in in vivo experiments with rats, and in in vitro studies, respectively.

Lee and Clydesdale (1979), however, showed that the baking procedure caused marked changes in the "iron profile" of added fortification iron. The fortification iron is not associated to any components in the dietary fiber complex, and would most probably go into solution during the baking procedure, resulting in different behavior than that of the naturally occurring iron in the bread.

Morris et al (1976) claimed that iron is associated mainly to phytate as monoferric phytate and is released by fermentation. Hallberg (1984), on the other hand, claimed that only 5 % were present as monoferric phytate.

IN VIVO EXPERIMENTS ON IRON AVAILABILITY FROM WHEAT BRAN IN PIGS

The aim of paper VII was to investigate the influence of wheat bran on iron availability, and to make a comparison between the absorption of naturally occurring iron from cereals and ferrous sulphate.

In the study it was not possible to distinguish between the effects of dietary fiber and phytic acid on bioavailability since these two components exist together in the bran. The aim of the study was not to study any particular inhibitory or promoting components, but to look at bran as a whole, including iron, dietary fiber, phytic acid and other components present in the bran, but not in refined cereal products.

From the results of studies in this field, it seems that there is a variable availability in animal and human models. The tables in the review chapter illustrate the controversy in this field.

Most studies have been done on rats and humans, and in contrast to humans, rats seem to enjoy a good bioavailability of iron from whole grain cereal products.

One should, however, bear in mind that most experiments on rats are long-term experiments, carried out on anemic animals, while the human studies are usually carried out on normal individuals with probably satisfactory iron stores.

In our experiments pigs were chosen as experimental animals because we wanted a model more similar to humans than rats.

Study VII was a long-term study using the hemoglobin regeneration technique on growing piglets.

Iron deficiency anemia was induced by feeding the piglets diets with very low iron content directly after birth, and in the repletion period the hemoglobin and serum iron levels were followed.

There was no difference in these parameters for pigs receiving 7 % bran in the diet and for pigs not receiving bran, but the same amount of iron in the form of ferrous sulphate. The pigs were fed these diets for up to 8 months, and no sign of anemia was observed.

In the second part of the study, one group of pigs received all their iron from cereals with an addition of 20 % bran, while the other group received nearly all their iron from ferrous sulphate and no bran. Neither in the repletion period, nor in the period after (up to 8 months) when the normal level of hemoglobin was reached, were any significant difference in the bioavailability of iron observed between the two diets.

In conclusion, bran did not seem to have any inhibitory effect on iron absorption, even at relatively high amounts (20 %). The iron naturally occurring in cereals seemed to be as available as ferrous sulphate when the pigs were repleting from an anemic state.

The results of our study are in accordance with the results of Sandberg (1982) showing even a slight increase in apparent iron absorption during 4 days of consumption of raw bran in ileostomy patients.

In a long-term study with bran added to the diet, a small, but significant decrease in serum calcium and an indicated decrease in iron absorption were shown after 7 weeks. These changes, however, disappeared after 35 weeks of bran addition (Andersson et al, 1979).

The contrast of our pig study to most of the human studies could be explained by species differences. There are, however, differences in results from experiments with the same species, indicating that other factors in the experimental design might be of greater importance, e.g. duration of the study and the subject's individual need of iron.

In anemic animals there is a need for the mineral in question. When there is a need it seems that the body regulates the absorption of the mineral, according to the body's need for the mineral. The mineral then seems to be available from unrefined cereal products, in spite of the presence of chelating agents, such as dietary fiber components and phytic acid. It may be that these components act as regulatory factors in the diet when the body's storage is satisfactory (Hallmans, 1983).

LONG-TERM ADMINISTRATION OF DIETARY FIBER TO DIABETICS

Much less is known about the mineral bioavailability in humans than in animals. Most of the studies done on humans are short-term studies, in which only a single meal has been given in order to study the mineral absorption. Few long-term studies have been published. It has, however, been claimed that an adaptation of mineral absorption will take place when a diet containing a high content of unrefined cereal products is given over a longer period (Walker et al, 1948; Campell et al, 1976; Bagheri et al, 1981).

Experiments with a duration of longer than three to four weeks show no change in the mineral absorption from whole grain cereal products (Widdowson et al, 1942; Walker et al, 1948; Heaton et al, 1974; Weinreich et al, 1977; Guthrie et al, 1978; Sandstead et al, 1978; Bagheri et al, 1981; Rattan et al, 1981; Robertson et al, 1981; Sandström et al, 1983).

Dietary fiber and diabetes

The hypothesis that a low-fiber diet is associated with diabetes was first published in 1975 by Trowell (Trowell, 1975), but it is still not fully documented. A number of investigations, however, have shown a favorable effect from fiber in diabetic patients (Jenkins et al, 1976; Kiehm et al, 1976; Simpson et al, 1979).

Dietary fiber could be given to diabetic patients either as food items with a natural high fiber content (e.g. vegetables and bread containing whole grain flour), naturally concentrated fiber sources (bran) or as isolated fiber components (guar gum, pectin).

The different fiber sources seem to have different effects. It has been shown that purified, viscous fibers, like guar gum and pectin can cause a stabilization of postprandial glycemia in both normal humans and diabetics (Jenkins et al, 1976, 1978a) and can reduce urinary glucose losses (Jenkins et al, 1978b). Guar gum has also been effective in long-term experiments (Jenkins et al, 1980; Aro et al, 1981).

Wheat bran gives only a small or no effect in short-term experiments (Wahlqvist et al, 1979; Hall et al, 1980). In long-term studies, however, a positive effect of bran on glucose tolerance was observed (Brodribb et al, 1976; Simp-

son et al, 1979a; Sandman et al, 1983). The mechanism of this effect is still not clear.

In natural high-fiber food items several components could have a positive effect on diabetes: the fiber itself, the structure of the fiber (Haber et al, 1977) or the combination of high-fiber - high-carbohydrate or trace elements present in the food items.

Treatment of diabetic patients with dietary fiber seems to be advisable, either in the form of isolated fiber components or as naturally occurring food items. It would, however, be preferable to give the fiber as part of the daily diet. Bread is eaten every day and could easily be included in every meal. The best way to include fiber in the diet of diabetic patients would be to give them whole grain bread available from regular bakeries or shops. In most studies isolated fiber components included in the bread have been the most common source of fiber supplementation to the patients. This kind of bread, however, is difficult to obtain as it has to be specially produced.

The purpose of the study described in paper VIII was to observe the influence of bran and guar gum, added to the bread, on mineral absorption in a long-term study of diabetic patients, and not primarily the medical aspect of diabetes. These two fiber additions were chosen because of their different abilities to associate to divalent cations. While bran contains chelating components such as phytic acid, guar gum, on the other hand, does not contain either phytic acid or other ionized groups. Pectin, however, was not chosen as the purified viscous fiber source because it contains uronic acid, which has been shown to influence calcium absorption (James et al, 1978).

From a technical point of view, it is easier to produce a whole grain bread with bran than a bread containing guar gum. A guar gum bread dough is difficult to handle because it is very sticky which causes problems in regular bakery equipment, and few bakeries would therefore be willing to produce such a bread.

Guar gum is available in particles of different sizes. We found in our pilot bakery that mixing guar gum with fairly large particle sizes with the flour before the addition of water gave bread of an acceptable quality which could be produced without any difficulties with up to 15 % of the flour replaced by guar gum. This is in contrast to other studies where bread with this concentration was found to collapse and the crumb structure show excessively large

holes (Apling et al, 1983). The reason why we succeeded with such a high content of guar gum could be the strong wheats used in Norway, which have the ability to carry high proportions of additional ingredients without losing their baking properties. The bread that was made with 15 % guar gum also had organoleptic properties acceptable to the patients.

In paper VIII, 28 insulin-dependent patients were followed through 3 dietary periods, each with a duration of three months. In the control period (first three months), all patients were given low-fiber diets, while in the next two periods bread enriched either with guar gum or bran was given.

Serum concentrations and urine excretion of the minerals were chosen as monitoring parameters. The reason for choosing these parameters was that in long-term experiments with patients, these are the only acceptable tests which could be repeatedly performed on the patients without causing them any undue discomfort.

From the study it appears that dietary supplementation with guar gum or wheat bran can improve the glucose metabolism in patients with diabetes. No changes could be observed in the serum concentrations of calcium, inorganic phosphate, magnesium, iron, copper, zinc or selenium. The urinary excretion of calcium, however, was slightly lowered during the bran period, whereas the other minerals measured were not affected. Except for this slightly lowered calcium excretion in the urine when given bran, the two different sources of fiber supplementation did not show any differences in the mineral absorption study.

It has been claimed that the zinc excretion in urine in diabetic patients is higher than normal (Pidduck et al, 1970; Hägglöf et al, 1983). In our study we were interested in comparing zinc excretion in diabetic patients with and without fiber supplementation, and even if the zinc excretion is somewhat higher in these patients, the relative differences, if there were any, could then be observed.

From this study it could be concluded that the availability of the minerals did not seem to be significantly affected, even with additional high amounts of either bran or guar gum in the diet.

Even with chelating agents, such as phytic acid and other dietary fiber components present in the bran, no inhibition of mineral utilization could be observed.

From this and other studies (e.g. Nygren et al, 1984) fiber-rich and carbohydrate-rich diets, which usually also contain high levels of minerals and trace elements, should be recommended for diabetic patients. Such a diet would positively affect the glucose tolerance, and could also be of importance for healthy people.

CONCLUSIONS

The conclusions of the studies in this thesis may be summarized as follows:

1. Considerable variations were found in the dietary fiber content of flours: the content in whole grain wheat flour varied from 11.9 % to 17.9 %, in whole grain rye flour from 13.9 % to 23.8 % and in white wheat flour from 2.8 % to 4.6 %. The fiber content of bran varied from 57.2 % to 62.2 % (Paper I).
2. Cereal products, bread not included, showed a great variation in the dietary fiber content, with contents sometimes under 10 % even if they were called "high fiber products". There are, however, products on the market with a fiber content of up to 50 % (Paper II).
3. A fairly wide variation was observed in the dietary fiber content in bread with the same trade name, but produced in different bakeries, having a variation from 20 - 65 % in the whole grain flour content. 80 % of the bread had a content of more than 35 %, with a content of 45 % being most frequent. A high content of whole grain flour seems to contribute positively to the sensory properties of the bread (Paper III).
4. The soluble fiber components in the dietary fiber complex were associated with considerable amounts of ash, while only minor amounts could be detected in the insoluble fiber fraction. During fermentation and breadmaking a decrease in the fiber associated with ash was observed. The total dietary fiber content, corrected for the ash, was not influenced by the baking procedure. Up to 75 % of the total phytate content was detectable in the soluble fiber fraction and was considerably reduced. Up to 60 % of the total iron was associated to components in the dietary fiber complex other than phytate. Only 10 % of the iron seemed to be associated to phytate. For the rest of the minerals studied (Ca, Zn, Mg), however, the main part of the fiber-associated minerals seemed to be bound to phytate, and were liberated during fermentation (Papers IV, V, VI).
5. In a bioavailability study of iron using anemic pigs as an experimental model, naturally occurring iron from wheat bran was found to be as available as ferrous sulphate, and bran was found to have no inhibitory effect on iron absorption, even when 20 % bran was added to the diet.

6. When giving diabetes patients either bran- or guar gum-enriched bread in a long-term experiment, no changes were found in the mineral concentration of either urine or serum except for a slightly lowered urinary calcium excretion during the bran period (Paper VIII).

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ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to:

Professor Nils-Georg Asp for his never-failing support and encouragement. He not only introduced me to the field of fiber, helped me through valuable discussions and criticism, he also made me continue at the most difficult times, when it seemed impossible to go on;

Professor Kaare R. Norum for his contagious enthusiasm in the field of nutrition, his never-ending inspiration and support, and for convincing me that there is always more energy to carry on;

Civ.ing. Olav Aasmundrud for his unfailing support, valuable discussions, and inspiring personality which always cleared one's mind;

Professor Thor Homb for valuable advice, discussions, and support;

Dr T. Drakenberg, Dr T.F. Schweizer, Dr A. Lysø, Civ.ing. G. Rognrud, Ing. B. Wilsher, Dr S. Vaaler, and Dr J. Aaseth for their helpful guidance and stimulating collaboration. Without them the thoughts and ideas would have been limited;

Dr A. Bjørneklett for valuable discussions and a lot of stimulation;

The National Institute of Technology in Norway for giving me the possibility to do this work, and to those of my colleagues and friends who supported me there;

Dr Carl Erik Albertsson who introduced me to the field of cereal chemistry;

Christina Dahlgren for her efficient secretarial work, and for her warm, supporting personality;

Kerstin Ulveland for typing the final manuscript;

All my friends and colleagues at the Departments of Food Chemistry and Applied Nutrition in Lund, for always taking care of me and sharing their knowledge with me;

All colleagues in Norway and elsewhere who contributed to this work and who supported me. I never knew there were so many helpful people in the world;

To everyone in the cereal branch in Norway and Sweden;

To all my friends for always being there when it really counted, what would I have done without them?

Special thanks are due to my parents who told me that if I really believed in something, I should go for it.

These studies were supported by grants from the Royal Norwegian Council for Scientific and Industrial Research. Financial support was also given by the mills, bakeries, and Grain Cooperation in Norway and by the National Swedish Board for Technical Development.



Dietary Fiber Content in Cereals in Norway

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ABSTRACT

Cereal Chem. 58(6):524-527

To investigate the content and variability of dietary fiber in cereals, whole-grain rye flour and four different milling fractions of wheat were collected from each of the nine mills in Norway. Soluble and insoluble dietary fiber was determined with an enzymatic method corresponding to the physiological definition of dietary fiber (sum of nondigestible polysaccharides and lignin). The average values of the different products

were 3.6% for wheat flour, 14.4% for whole-grain wheat flour, 52.2% for wheat bran type 1 (including both outer layers and germ), 59.3% for wheat bran type 2 (almost only outer layers), and 16.8% for whole-grain rye flour. Considerable variation was found in the dietary fiber content of flours from different mills, whereas that of brans was almost constant.

Diet, nutrition, and health are connected. Even though the diet in Norway must be considered nutritionally adequate, generally covering the required essential nutrients, significant negative changes have occurred over the last 75 years (Anonymous 1975). One reason is that the consumption of cereals and potatoes has declined drastically. Consequently, carbohydrate content in the diet has decreased and the carbohydrate composition has changed. The decrease has been particularly great for starch. The

consumption pattern, as elsewhere in the Western countries, has tended toward more refined food. Not only are Norwegians consuming less cereals, but the cereal products are, to a larger extent, made from refined white flour, depleted in dietary fiber and in essential nutrients like vitamins and minerals.

In recent years, the importance of dietary fiber in the diet has been extensively investigated (Kay and Strasberg 1978, Kelsey 1978). Accumulating evidence indicates that dietary fiber constituents have several important beneficial effects. Fiber is a well-recognized remedy for constipation, but it also has prominent effects upon lipid and carbohydrate metabolism that might be of importance in the prevention of atherosclerotic diseases and diabetes. Colonic cancer is another "Western" disease in which lack of dietary fiber has been suggested to be one pathogenetic factor. In Norway, two considerable changes in dietary fiber intake have

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occurred recently. First, the total intake has been reduced to about half during the last 70 years (Trygg 1976). Second, the fiber composition has changed. Earlier, cereals were the prominent source of fiber, whereas today fruit and vegetables are the main contributors. The same development is true for Great Britain (Bingham et al 1979). This is significant because fiber from different sources has different physiological properties.

For many, bran has become almost synonymous with fiber. It is the most concentrated form of fiber and is readily and cheaply

available to the public from a variety of commercial sources. It is also a well-studied product, for most investigations on physiological effects have been made with bran as the source of fiber. In addition to its high fiber content, bran is also a good source of minerals and vitamins.

Cereal fiber intake could easily be increased by greater consumption of unrefined cereal products. The optimal amount of daily fiber intake has not yet been defined, however.

Knowledge about dietary fiber content in foodstuffs is interesting from a consumer's point of view. The definition and terminology of fiber are still under discussion. This is one reason for disagreement concerning the analysis of fiber. The crude fiber method, measuring only a small and variable fraction of the total dietary fiber, has now generally been abandoned (Southgate 1975, Van Soest and Robertson 1976). The neutral detergent fiber method, with amylase treatment, is considered to give a satisfactory measurement of insoluble components but does not measure water-soluble components such as pectin, gums, and some hemicelluloses (Southgate et al 1978). Fractionation methods with colorimetric or gas chromatographic quantification of dietary fiber constituents (Southgate 1976, 1977; Theander and Åman 1979) give important detailed information but are too laborious for routine purposes.

This article gives information on the fiber content in various cereal products as analyzed by an enzymatic gravimetric procedure corresponding to Trowell's definition of dietary fiber as the sum of undigestible polysaccharides and lignin (Trowell et al 1976).

MATERIALS AND METHODS

Norway, where the extraction rate is under governmental control, has a total of nine mills. Wheat was of primary interest in this study, rye to a lesser extent. The following samples were collected from each mill: 1) wheat flour (78% extraction, which is normal in Norway); 2) whole-grain wheat flour; 3) wheat bran type 1, defined as "millrun," the material left over after the milling process (22% of the whole grain, containing outer layers and germ); 4) wheat bran type 2, commercially found in markets and bakeries (about 15% of the whole grain, containing mostly outer layers); and 5) whole-grain rye flour.

Sample Preparation

The samples did not contain more than about 5% lipids and were therefore not defatted (Schweizer and Würsch 1979). The samples were ground in a Cyclotec sample mill (Tecator) to a particle size of less than 0.45 mm.

General Methods

Ash was determined by ignition at 550–600°C to constant weight (6 hr). The dry weights were determined by oven drying at 105°C to constant weight (18 hr). Unless otherwise indicated, weights and yields are stated on a moisture free basis.

Dietary Fiber Method

The method used to determine the fiber content was based on Hellendoorn's enzymatic method (Asp and Johansson 1981,

TABLE I
Dietary Fiber Content of Cereals*

Sample No.	Dietary Fiber (%)		
	Insoluble	Soluble	Total
Wheat Flour			
1	2.3	0.5	2.8
2	2.5	0.7	3.2
3	3.8	0.8	4.6
4	2.6	0.4	3.0
5	3.5	0.8	4.3
6	2.7	1.1	3.8
7	2.7	1.4	4.1
8	2.7	1.2	3.9
9	2.1	1.0	3.1
Mean ± SD	2.8 ± 0.5	0.8 ± 0.4	3.6 ± 0.6

Whole-Grain Wheat Flour

1	12.6	1.3	13.9
2	13.3	1.5	14.8
3	11.3	1.0	12.3
4	17.5	0.4	17.9
5	11.8	1.8	13.6
6	10.8	1.1	11.9
7	11.2	0.8	12.0
8	15.1	1.8	16.9
9	15.1	1.3	16.4
Mean ± SD	13.2 ± 2.3	1.2 ± 0.5	14.4 ± 2.3

Wheat Bran Type 1 (Millrun)

1	48.6	3.5	52.1
2	47.7	3.8	51.5
3	50.1	2.8	52.9
Mean ± SD	48.8 ± 1.2	3.4 ± 0.5	52.2 ± 0.7

Wheat Bran Type 2 (Commercial)

1	54.5	2.7	57.2
2	53.5	3.8	57.3
3	55.6	3.7	59.3
4	59.0	3.2	62.2
5	54.0	3.3	57.3
6	56.9	3.8	60.7
7	57.1	3.7	60.8
8	55.4	4.0	59.4
Mean ± SD	55.8 ± 1.8	3.5 ± 0.4	59.3 ± 1.9

Whole-Grain Rye Flour

1	12.4	2.2	14.6
2	12.3	2.6	14.9
3	15.8	2.4	18.2
4	15.8	1.1	16.9
5	15.5	2.4	17.9
6	11.3	2.6	13.9
7	13.7	3.3	17.0
8	12.4	1.6	14.0
9	18.2	5.1	23.3
Mean ± SD	14.2 ± 2.3	2.6 ± 1.1	16.8 ± 3.0

* Moisture free basis, corrected for protein, starch, and ash.

TABLE II
Ash Content and Total Fiber Content in Different Wheat Flours

Wheat Flour No.	Ash Content ^a	Total Fiber ^a
1	0.63	2.8
2	0.54	3.2
3	0.66	4.6
4	0.45	3.0
5	0.48	4.3
6	0.43	3.8
7	0.58	4.1
8	0.64	3.9
9	0.64	3.1
Mean ± SD	0.56 ± 0.09	3.6 ± 0.6

^a Percent of moisture-free flour.

Hellendoorn et al 1975) with the following modifications. The *in vitro* enzyme digestion was performed with pepsin at pH 1.5 for 1 hr and with pancreatin at pH 6.8 for 1 hr, thus simulating the conditions in the human gastrointestinal tract. Insoluble fiber components were recovered by filtration and soluble components by ethanol precipitation followed by filtration. The filtrations were carried out with Tecator's Fibertec system (Tecator AB, Höganäs, Sweden), using about 0.5 g of celite as a filter aid. The insoluble and soluble dietary fiber fractions thus recovered were dried overnight at 105°C before weighing. They were then analyzed for residual nitrogen by the Kjeldahl method (conversion factor to protein = 6.25). Remaining starch was also determined by measuring glucose following extensive glucoamylase digestion. Glucose was assayed with a TRIS-glucose oxidase reagent (Dahlqvist 1961). Starch, including dextrans and maltose, was assayed by measuring the increase in free glucose after incubation with γ -amylase (amylglucosidase, Boehringer-Mannheim). Ash was determined as described above.

The dietary fiber values reported in the tables are the means of triplicate gravimetric analyses, corrected for remaining traces of starch, protein, and ash.

RESULTS AND DISCUSSION

Dietary fiber content by the modified Hellendoorn's method is shown in Table I. The average values were 3.6% for wheat flour, 14.4% for whole-grain wheat, 52.2% for bran type 1, 59.3% for bran type 2, and 16.8% for rye flour.

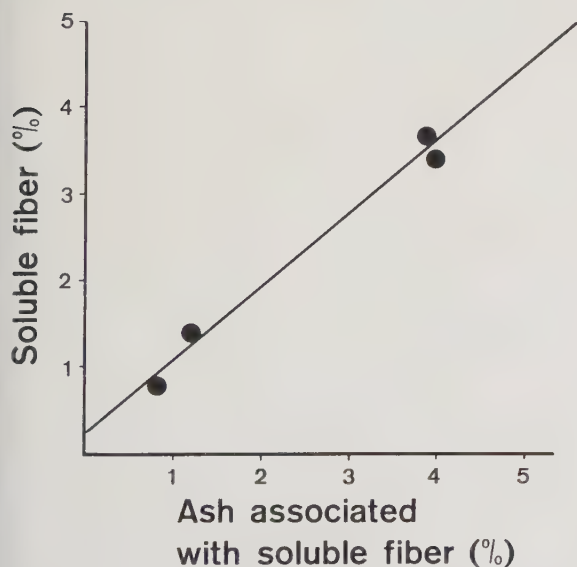


Fig. 1. Relation between soluble dietary fiber (corrected for ash) and the ash associated with it in four wheat products (percent of product on moisture free basis).

One of the advantages of this method, as compared with other gravimetric procedures, is that it measures soluble as well as insoluble components. The soluble fraction contributed 6–20% of the total fiber. This is probably the main reason that our figures are somewhat higher than usually found with other gravimetric methods (Anderson and Clydesdale 1980; Saunders and Mantala 1977, 1979).

Practically no data are available concerning the variability of dietary fiber content in cereals. In our materials, the two kinds of bran from different mills had very similar dietary fiber content. Considerable variations were found for whole-grain flours and also for wheat flours. The total dietary fiber in whole-grain wheat flour varied from 11.9 to 17.9% and that in whole-grain rye flour from 13.9 to 23.3%. The main reason for this may well be that Norway imports about 80% of its grain and that grain batches from different countries are mixed. Flours from the same mill probably vary in dietary fiber content from time to time. For food labeling, therefore, using an average value from all the mills for each type of flour is probably best.

The variation in dietary fiber content in white flours could also reflect actual variations in extraction rate. In Norway, this is supposed to be 78% and is adjusted according to the ash content. Nevertheless, differences were found in ash content from mill to mill, probably from technical adjustments during processing. No correlation existed between ash content and total dietary fiber content in our samples of wheat flour (Table II), indicating that the dietary fiber content of wheat flour cannot be predicted simply from the ash content.

The ash contents of the insoluble and soluble fiber fractions were also determined. The insoluble fraction, constituting 70–90% of the total dietary fiber, did not contain measurable amounts of minerals (ash). The soluble fiber fraction, on the other hand, contained minerals in amounts equal to the total mineral content of the product. A linear correlation was found between the amount of soluble fiber and the ash associated with it (Table III, Fig. 1). The nature of this association between soluble fiber and ash, also found by Schweizer and Würsch (1979), is under investigation.

Dietary fiber values were corrected for ash and for the residues of protein and starch remaining after enzyme digestion. The protein residues in the insoluble fraction of the wheat bran were close to 2% of the bran. Half of this, however, was due to the added enzymes, as shown by running an enzyme blank. The remaining 1%, corresponding to 2% of the dietary fiber, is probably truly indigestible protein associated with the fiber. In the soluble fraction, the corresponding protein residue (above the blank) was about 0.5%, corresponding to 10% of the water-soluble fiber. Whether nondigestible protein should be included as part of dietary fiber is an unresolved question. Saunders and Betschart (1980) recently claimed that protein is a component of dietary fiber. Our data, however, indicate that the fiber-associated, nondigestible protein is much lower than suggested by these authors (10%, versus a maximum of 3% according to our data). In the wheat flour fiber, almost no protein was detectable (above the blank), whereas in whole wheat flour, the residue amounted to about 3% of the total dietary fiber.

A starch residue of about 1% was found in all the flours but not in the brans. This residue, determined by extensive glucoamylase treatment, can be eliminated by including an additional step in the

TABLE III
Dietary Fiber Content^a (Corrected for Protein, Starch, and Ash) and Fiber-Associated Ash in Various Wheat Products

	Wheat Bran				Flour			
	Type 1 ^b		Type 2		Whole-Grain		Extracted	
	Fiber	Ash	Fiber	Ash	Fiber	Ash	Fiber	Ash
Total dietary fiber	52.2 ± 0.7	4.0 ± 0.2	59.2 ± 1.5	4.0 ± 0.1	14.4 ± 2.0	1.3 ± 0.1	3.7 ± 0.6	0.7 ± 0.1
Insoluble fiber	48.8 ± 1.1	0	55.6 ± 1.4	0	13.2 ± 1.9	0	2.9 ± 0.4	0
Soluble fiber	3.4 ± 0.4	4.0 ± 0.2	3.6 ± 0.4	4.0 ± 0.1	1.2 ± 0.4	1.3 ± 0.1	0.8 ± 0.3	0.7 ± 0.1

^a Mean ± standard deviation in percent of moisture-free material from nine different mills, each analyzed in triplicate.

^b Obtained only from three mills.

final modification of the enzymatic fiber method.³

In conclusion, a gravimetric assay of soluble and insoluble residues following enzyme digestion gives an accurate and rapid estimate of dietary fiber content. The dietary fiber content of flours from different mills varied considerably, whereas that of brans was almost constant. Because of Norway's extensive importation of grain, the mixing of different grain batches, and the resulting variation in the flour produced, flour from the same mill may vary in dietary fiber content from time to time.

For food labeling, therefore, an average dietary fiber content from all the mills for each type of flour will probably be most adequate.

¹N.-G. Asp., C.-G. Johansson, H. Hallmer, M. Siljeström. 1981. Unpublished data.

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[Received January 2, 1981. Accepted May 29, 1981]



NOTE

Dietary Fiber Content of Different Cereal Products in Norway

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Cereal products are important sources of dietary fiber, and an easy way to increase the content of dietary fiber in the diet is to increase the consumption of unrefined cereal products. These products are also important sources of minerals and vitamins.

Information about the content of dietary fiber in foodstuffs is of interest to consumers. In most food tables, fiber values are lacking or inaccurate because of use of the crude fiber method. However, this method has generally been abandoned (Southgate 1976, Van Soest and Robertson 1976). The latest edition of the British food tables, however, includes dietary fiber values according to Southgate's method (Paul and Southgate 1978).

The definition and terminology of dietary fiber are still under discussion, but authors seem to agree that all nonstarch polysaccharides and lignin should be included. Some authors (Saunders and Betschart 1980) prefer to include undigestible protein as well. Other substances associated with dietary fiber, such as phytic acid and tannins, are generally quantitatively small.

Routine purposes require the use of a rapid and accurate method for determining dietary fiber. Fractionation methods with colorimetric or gas chromatographic assays of monomer constituents give important detailed information but are too laborious for routine purposes. The neutral detergent fiber method gives satisfactory measurements of insoluble components but does not measure soluble components, which in a mixed diet could account for up to 40% (Asp and Johansson 1981).

This article gives information on the fiber content in various cereal products analyzed by an enzymatic, gravimetric procedure (Asp et al 1982). The method measures the residue after enzymatic removal of protein and starch, corrected for undigestible protein and ash associated with the fiber.

The contents of the most commonly used cereals, wheat and rye, were reported previously (Frølich and Asp 1981), but means are included in the present paper for comparison.

MATERIALS AND METHODS

Cereal products were bought in local markets in Norway, except for the flours that were obtained directly from the mills. The products were then divided into four main groups: flours obtained throughout the year by The Norwegian Grain Cooperation from each of Norway's nine mills; ready-to-eat cereals, both Norwegian and foreign types; crackers, flatbread, and crispbread; and rice

and pastas.

Sample Preparation

Samples containing more than 10% fat were defatted with hexane or with chloroform-methanol (2:1, v/v) in a Soxhlet apparatus before use (Schweizer and Würsch 1979). The samples were ground in a Cyclotec sample mill (Tecator) to a particle size of less than 0.45 mm.

General Methods

Ash was determined by ignition at 550–600°C to constant weight (6 hr). The dry weights were determined by oven-drying at 105°C to constant weight (18 hr).

Dietary Fiber Method

The method used to determine the fiber content was based on Hellendoorn's gravimetric, enzymatic method (Hellendoorn et al 1975) with the following modifications, described by Asp et al 1982. To remove starch completely, an extremely heat-stable α -amylase (Termamyl 60 L, Novo, Copenhagen, Denmark) was used in an initial gelatinization step at 100°C for 15 min. Enzyme digestions were performed with pepsin at pH 1.5 for 1 hr and with pancreatin at pH 6.8 for 1 hr, both at 40°C, thus simulating the conditions in the human gastrointestinal tract. Soluble dietary fiber components were precipitated with ethanol, recovered together with the originally insoluble components by filtration. Separate values of insoluble and soluble fiber can be obtained by filtration in two steps: initial filtration recovering the insoluble components, and another filtration after alcohol precipitation of the soluble components. These two procedures give the same values of total dietary fiber (Asp et al 1982). The filtrations were performed with Tecator's Fibertec system (Tecator AB, Höganäs, Sweden), using 0.5 g of Celite as a filter aid. The fiber filtrates recovered were dried overnight at 105°C before weighing. They were then analyzed for ash and residual nitrogen by the Kjeldahl method (conversion factor to protein = 6.25). Dietary fiber values given are corrected for residual protein and ash.

The dietary fiber values reported in the tables are the means of duplicate gravimetric analyses. The standard deviation of total dietary fiber calculated from duplicate samples was previously determined to 0.32 g/100 g in samples with low to moderate dietary fiber content (Asp et al 1982). All values are given in percent, both on a moisture-free basis and on a wet-weight basis, and represent means of duplicate analysis.

RESULTS AND DISCUSSION

The contents of dietary fiber for the different cereals on the market in Norway are shown in Tables I–IV.

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² W. Frølich, 1982.

TABLE I
Dietary Fiber Content of Flours and Other Milling Products

	Dietary Fiber (wet weight, %)	Moisture (%)	Dietary Fiber (dry weight, %)
White wheat flour (78–80% extraction) ^a	3.2	11.2	3.6
Whole grain flour, wheat ^a	12.6	11.4	14.4
Barley flour (50% extraction)	10.8	10.7	12.1
Barley flour (70% extraction)	13.6	10.4	15.2
Barley, whole grain	23.2	8.5	25.3
Whole grain flour, rye ^a	14.7	11.4	16.8
Rye flour (mixed with 15% white wheat flour)	7.2	10.9	8.1
Oat flakes	10.7	8.6	11.7
Bran of wheat ^a	52.8	11.4	59.3
Bran-germ of wheat	22.1	8.1	24.0
Germ of wheat	19.7	9.1	21.7
Semolina	2.3	11.7	2.6
Triticale	13.9	7.4	15.0

^a From Frølich and Asp 1981.

TABLE II
Dietary Fiber Content of Ready-To-Eat Breakfast Cereals

Cereal	Dietary Fiber (wet weight, %)	Moisture (%)	Dietary Fiber (dry weight, %)
Kellogg's Corn Flakes	6.2	5.6	6.6
Kellogg's Müsli	12.8	8.3	14.0
Kellogg's Rye Flakes	11.2	11.7	12.7
Kellogg's Oat Mix	10.1	10.4	11.3
Kellogg's Bran Buds	29.5	7.5	31.9
Kellogg's Fiber Rich	33.8	7.5	36.6
OTA, Sun-Breakfast	7.6	8.4	8.3
Collett's Everyday (Norwegian)	9.8	6.1	10.2
Bjølens 4 grain (Norwegian)	10.0	8.0	10.9

TABLE III
Dietary Fiber Content of Crackers, Flatbread,
and Crispbread

Product	Dietary Fiber (wet weight, %)	Moisture (%)	Dietary Fiber (dry weight, %)
Crackers			
Bran cracker, type I (G. Gundersen)	45.8	4.4	47.9
A/S Sætre, type II	34.9	2.9	35.9
Bran cracker type III (Hoba A/S)	42.0	3.5	43.5
Bran cracker, health shop	36.6	4.9	38.5
Flatbreads and crispbread			
Korni Flatbread	17.9	3.4	18.5
Korni Export Flatbread	17.9	3.4	18.5
Korni Breakfast Flatbread	18.6	3.0	19.2
Korni Flatbread with bran	22.0	4.2	23.0
Ideal Flatbrød	7.6	3.0	7.8
Ideal Homemade	10.7	3.2	11.1
Ideal Fiber flatbread	15.7	3.7	16.2
Korni, Thin Crispbread	21.9	4.8	23.0

Table I shows the dietary fiber content of different flours and other milling products, most of which have a fiber content of 11.7–59.3% (dry basis). The content of soluble fiber components in oat is considerably higher (50%) (unpublished data²) than in other cereals (7–20%) (Frølich and Asp 1981).

Table II shows the dietary fiber content of ready-to-eat cereals. The products can be divided into two groups with respect to dietary fiber content, one based on bran and the other on whole grain flour. Some of these products are extremely rich in fat and sugar and should not be regarded as healthful, high-fiber products.

Flatbread, a Norwegian specialty, is produced primarily from whole grain flour and has a dietary fiber content of approximately 22% (Table III).

TABLE IV
Dietary Fiber Content of Pasta and Rices

Product	Dietary Fiber (wet weight, %)	Moisture (%)	Dietary Fiber (dry weight, %)
Sopps, spaghetti	4.1	9.6	4.5
Sopps, macaroni	4.5	8.9	5.0
Sopps, whole wheat flour spaghetti	9.7	8.7	10.7
Sopps, whole wheat macaroni	10.8	8.1	11.8
Galla, porridge rice	3.0	10.0	3.3
Galla, precooked porridge rice	2.3	11.2	2.6
Galla, precooked rice	3.5	8.6	3.9
Galla, party rice	4.6	10.2	5.1
Galla, dinner rice	3.7	10.6	4.1
Ming, precooked rice	3.3	8.9	3.6
Ming, American rice	3.0	9.8	3.4
Ming, porridge rice	2.1	11.4	2.3
Geisha, parboiled rice	3.1	7.8	3.4
Geisha, porridge rice	2.1	9.2	2.3
Geisha, dinner rice	3.2	11.2	3.6
Geisha, nature rice	4.6	10.7	5.1
Unpolished, round grain rice (health shop)	3.4	6.8	3.7
Unpolished, long grain rice (health shop)	4.2	11.6	4.7
Nature rice, biodynamic-grown (health shop)	4.5	10.2	5.0
Uncle Ben, parboiled rice	2.9	7.9	3.1
Uncle Ben, brown rice, type I	9.1	10.2	10.1
Uncle Ben, brown rice, type II	8.7	10.3	9.7

Bran crackers have become increasingly popular in Norway and are a more acceptable way of eating bran for the consumer.

Pasta products in Norway are based either on wheat flour (78% extraction rate) or on whole wheat flour. The whole flour products have a fiber content about twice as high as that of the other pasta products (Table IV).

The content of dietary fiber in rice is rather low (Table IV), in most cases lower than that of Norwegian white wheat flour (78% extraction rate). Even rice bought in health shops and advertised as unpolished has a rather low fiber content, not much higher than typical rice on the market. Only two types of rice have a higher dietary fiber content than others, indicating more unrefined products.

The values given led us to conclude that products with a relatively high content of dietary fiber are on the market. This information is important to consumers who require increased dietary fiber content.

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[Received May 26, 1982. Accepted September 28, 1982]



WHOLE GRAIN FLOUR CONTENT AND SENSORY PROPERTIES IN ONE VARIETY OF NORWEGIAN BREAD

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SUMMARY

Bread from 24 bakeries in Eastern and Western Norway were analysed for the nutritional properties iron, thiamin, dietary fiber and for sensory properties.

The results show a considerable difference in content of dietary fiber and iron, a smaller difference in content of thiamin. There is significant correlation between content of dietary fiber and thiamin. Results also indicates that whole grain flour content in Kneipp bread has increased over the past five years.

Sensory properties were correlated to content of dietary fiber. Significant correlation was found between fiber and impression of crust and crumb, texture of crust and flavour.

INTRODUCTION

It is part of Norwegian nutrition policy to increase grain consumption, specially products containing whole grain flour. One way to achieve this would be through a higher bread consumption, it is therefore important that the market offers a variety of bread that suit consumers preferences.

A consumer study (8) shows that the consumers consider nutritional value as an important quality factor of bread, and associate high nutritional value with a high content of whole grain flour, e.g. dark bread. Such bread was also often preferred because of its taste. Thus it is important to the consumers that a good selection of dark bread is available, and that they are given the necessary information of the products as background for their choice when buying bread.

The whole grain content is important information for the consumer, but only a few bread products have labeling showing this. The most widely used variety of bread containing whole grain flour is the Kneipp bread. A previous survey has shown widely varying whole grain flour content between bakeries. (9). The aim of the present study was to determine the actual content in Kneipp bread from different bakeries by means of chemical analysis.

With increasing extraction rate, the content of dietary fiber, protein, fat, ash, most of the minerals and some vitamins will also increase. Of these nutrients, dietary fiber is the most suitable parameter to be used as a measure for whole grain flour. This is among others due to the relatively large differences in the fiber content between whole grain flour and white flour. Other parameters e.g. ash could also be used. One must on the other hand be aware that the correlation between ash content and whole grain flour, could to a great extent be altered by minerals added for enrichment reasons. The same is true for salt added during e.g. breadmaking.

The sensory properties of bread are important for the consumer preference. If the goal of increased bread consumption and particularly of dark bread, should be achieved, it is a necessity that these varieties have satisfactory sensory properties. Norwegian consumers claim that they prefer darker bread because of taste (8). An increase of whole grain flour in bread should

preferably increase the consumers preference for the products. It is also an objective of the present study to investigate a possible correlation between content of whole grain flour and different sensory properties.

MATERIALS

Bread of the Kneipp Variety was collected from 24 bakeries, 12 large industrial bakeries and 12 small. 12 bakeries were located in Eastern Norway, 12 in the Western part of the country. Loaves were collected from each bakery on 3 different days, to allow for daily variation in the production.

The samples were collected from the bakeries within one hour after baking, wrapped in paper bags and shipped to the Institute. Samples from the Western areas were shipped by air, in closed cardboard boxes. Samples from the Eastern areas were transported by car in open boxes, which allowed the steam to escape. All samples arrived at the Institute within 5-6 hours.

The bread samples were approximately 800 g size.

METHODS

Sample preparation

Each bakery sample consisted of 3 loaves. Each loaf was divided into 2 equal halves, one of these was stored in a deep freezer as a reserve. The other half was sliced in approx. 1 cm thick slices and every third slice of the three half loaves, were combined to one analytical sample of approx. 400 g. The samples were weighed accurately, dried on a tray at room temperature for 3-5 days, crushed in a Kenwood blender, and ground in a Tecator Cyclostyle Mill with 1 mm sieve. The samples were stored in closed jars at room temperature.

Chemical methods

Moisture

Moisture was determined by a method described by Nordic Committee for Food Analyses. (7). The sliced bread samples were weighed and airdried as described above. The remaining moisture was determined by drying at 130 °C for 2 hrs.

Dietary fiber

The method used to determine dietary fiber content, was based on Hellendoorn's gravimetric, enzymatic method, (3) described by Asp et al (2). To remove starch completely, an extremely heat-stable enzyme, amylase (Termamyl 60L, Novo, Copenhagen, Denmark) was used in an initial gelatinization step at 100 °C for 15 min. Further enzyme digestions were performed with pepsin (Merck no 7190) at pH 1.5 for 1 hr. and with pancreatin (Sign no P-1750) at pH 6.8 for 1 hr., both at 40 °C. Soluble dietary fiber components were precipitated with ethanol, recovered together with the insoluble fiber components with filtration, in a Tecator Fibertec System (Tecator AB, Höganäs, Sweden). Dietary fiber values given are corrected for residual protein (Kjeldahl Method) and ash. The dietary fiber values reported in the table are means of duplicate gravimetric analysis. The values are given on a moisture free basis.

Iron

Dry ashing was done on an aliquot of the predried material, at 450 °C for 18 hrs. Deionized water was added and the sample heated at 450 °C for another 2 hrs in order to remove traces of carbon material. The ash was dissolved in 0.1 N HNO₃, diluted and measurement of iron carried out in a Perkin Elmer Modell 2380 atomic absorption spectrometer, at the conditions recommended by Perkin Elmer. (6).

Thiamin

Thiamin was analyzed by the method recommended by ICC (5). The sample was hydrolyzed by 0.1 N HCl and Taka Diastase. Thiamin was purified on a Thiochrome Decalso column, oxidized to thiochrome by means of $K_3Fe(CN)_6$ in alkaline solution, and the fluorescence of the thiochrome was measured in a Perkin Elmer Fluorescence Model 203 Spectrophotometer.

Sensory methods

The sensory analyses were carried out by a trained laboratory panel of 8 to 10 judges. The method used was a scoring method (1) where nine different properties were evaluated on a 7 point scale. The analyses were carried out on the same day that the loaves were baked. The judges were given half a loaf each to evaluate total impression of the crust and the crumb, evenness of the surface as well as texture and flavour.

Statistical methods

Statistical evaluation of differences between variables was made by Bonferroni test.

RESULTS AND DISCUSSION

Content of dietary fiber

The content of dietary fiber in all samples is shown in table 1, given as a percent on basis of fresh weight and of moisture free basis. The range is 5,6% to 9,3% calculated on the moisture free basis, showing considerable variations in bread from the 24 bakeries. This expresses a difference in fiber-rich ingredients in the various recipes, even within bread that is supposed to be of the same variety. As mentioned 3 different samples

of bread were analysed from each bakery. However, there were no significant differences in dietary fiber content between these samples, indicating that each bakery reproduced their recipes well.

Table 2 shows the mean fiber content for bread from small and large bakeries, and for bread from the two different geographical regions. There was no significant statistical variation in fiber content between bread from the two types of bakeries, or from different geographical regions, although bread from the Western area had a lower mean value.

From a nutritional point of view, the whole grain content of bread should be increased, and not only the dietary fiber content. It is therefore important to give consumers information on whole grain content.

A linear correlation between dietary fiber content and the whole grain flour content has been shown (4). Dietary fiber content seems to be higher in bread than in the corresponding flours. (4).

This is probably due mainly to the resistant starch, which is formed during the baking process. It is therefore important to use a standard curve based on baked bread when correlating the content of dietary fiber to the content of whole grain flour. When using a standard curve based on flour, the formation of resistant starch etc. must be taken into consideration.

In this paper we have estimated the content of whole grain flour in the bread from a standard curve made on the basis of baked bread (4). The content of dietary fiber in baked bread was plotted against the content of whole grain flour. The dietary fiber values are corrected for undigestible protein and ash. The dietary fiber in the different bread samples are determined with the method of Asp et al (2), and the content of whole grain flour are then calculated from the standard curve. (See table 1.)

The data show a large variation in content of whole grain flour from 20% to 65%, indicating considerable differences between bakeries. This agrees with data found in a survey carried out in 1979/80 (9), where Kneipp bread recipes from a large number of bakeries were collected, and the proportion of whole grain flour were calculated. The content varied between 20% and 70%. However, in that study 30% was most frequently used, whereas the value is 45% in the present study. The two results cannot, however be compared directly since they are achieved by two different methods. Still it indicates that whole grain flour content in Kneipp bread has increased, in accordance with the nutritional goal of increased whole grain consumption.

As there is no enrichment of flour in Norway of minerals or vitamins, the content of other nutrients like iron and thiamin will also increase with the content of whole grain. The content of thiamin and iron in the bread samples were also determined as shown in table 2. The standard deviation is fairly large for iron, smaller for thiamin, reflecting the spread in the content of the nutrients in the different samples.

The data were tested for correlation between the content of dietary fiber and of thiamin and iron. As shown in table 3 the correlation to thiamin is highly significant, but there is no significant correlation to iron at the 95%-level. Tested at 90%-level however, significant correlation was shown.

In an analytical sample of bread, showing a high dietary fiber content, we could therefore also expect a high content of thiamin, providing there has been no enrichment of either components. This may be one way of estimating the thiamin content when the dietary fiber content is already determined, eliminating a costly analysis. However, this relationship need to be studied further before being put into practical use.

Sensory properties correlated to dietary fiber

Nine different sensory properties were evaluated, using a seven point scale. These properties have previously been shown to be important in characterizing Kneipp bread (10). Mean values and the standard deviation is shown in table 4. Total impression of both crust and crumb were evaluated as "good", the cutting surface as "even". Mean value for the texture of the crumb was between "slightly crisp" and "slightly soft". The properties describing texture were firm/crumbly and dry/doughy. Results showed that the mean value was close to the middle of the scale for both properties. The flavour was characterized as "distinct bread flavour".

The results showed low scores both for salt and sweet taste.

One would expect that the sensory properties of bread would be related to the content of whole grain flour. The results for the different sensory properties were therefore correlated to the content of dietary fiber, as shown in table 5. A significant and positive relationship was found between fiber content on the one hand and total impression of crust and crumb, texture of crust and flavour on the other hand.

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Table 1. Dietary fiber content and content of whole grain flour in Kneipp bread from different bakeries in Norway.

Bakery number	Bakery size	Dietary fiber wet weight, %	Dietary fiber dry matter, %	Content of whole grain flour, %
Eastern Norway				
No. 1	small	6,0	9,3	65
No. 2	"	4,7	7,3	40
No. 3	"	4,8	7,4	40
No. 4	"	5,1	7,8	45
No. 5	"	4,0	6,3	25
No. 6	"	4,8	7,6	45
No. 7	large	4,7	7,5	45
No. 8	"	5,8	9,2	65
No. 9	"	4,9	7,8	45
No. 10	"	5,2	8,1	50
No. 11	"	4,8	7,3	40
No. 12	"	4,8	7,4	40
Western Norway				
No. 13	small	3,6	5,6	20
No. 14	"	5,8	8,7	55
No. 15	"	4,5	7,2	40
No. 16	"	4,8	7,6	45
No. 17	"	4,9	7,7	45
No. 18	"	5,3	8,1	50
No. 19	large	4,6	7,1	35
No. 20	"	3,9	6,2	25
No. 21	"	5,3	8,2	50
No. 22	"	5,5	8,5	55
No. 23	"	4,6	7,0	35
No. 24	"	3,7	5,6	20
MEAN		4,9	7,5	45

Table 2. Content of some components in Kneipp Bread from different bakeries in Norway, (given pr. 100 g)

	Numbers of bakeries	Moisture, g	Dietary fiber, g	Iron, mg	Thiamin, mg
All bakeries	24	35,5 $\pm$ 1,3	4,9 $\pm$ 0,7	1,8 $\pm$ 0,4	0,13 $\pm$ 0,02
Eastern Norway	12	35,7 $\pm$ 1,4	5,9 $\pm$ 0,7	1,8 $\pm$ 0,3	0,13 $\pm$ 0,01
Western Norway	12	35,4 $\pm$ 1,2	4,7 $\pm$ 0,8	1,9 $\pm$ 0,5	0,13 $\pm$ 0,02
Small bakeries	12	35,4 $\pm$ 1,1	4,9 $\pm$ 0,7	1,9 $\pm$ 0,2	0,13 $\pm$ 0,02
Large bakeries	12	35,7 $\pm$ 1,0	4,8 $\pm$ 0,6	1,8 $\pm$ 0,2	0,13 $\pm$ 0,02

Table 3. Correlation between content of dietary fiber and other nutrients

Nutrient	Correlation coefficient
Thiamin	0,411 ^{***}
Iron	n.s. (Significant p:0,07)

n.s. = non significant correlation

* - $p < 0,05$

** - $p < 0,01$

*** - $p < 0,001$

Table 4. Sensory properties of Kneipp Bread from
different bakeries in Norway

Number of bakeries	24		
Properties:	Scores		Scale 7-1
Total impression of crust	5,15 $\pm$ 0,51		very good/very poor
Total impression of crumb	5,11 $\pm$ 0,48		very good/very poor
Evenness of slice surface	4,85 $\pm$ 0,64		even/uneven
Texture of crust	4,36 $\pm$ 1,05		crisp/soft
Texture of crumb	4,07 $\pm$ 0,69		firm/crumblly
- " -	3,74 $\pm$ 0,49		dry/doughy
Flavour	4,51 $\pm$ 0,59		very distinct/none
Salt taste	2,82 $\pm$ 0,71		very distinct/none
Sweet taste	2,15 $\pm$ 0,40		very distinct/none

Table 5. Correlation between content of dietary fiber and sensory properties

Sensory property	Correlation coefficient
Total impression, crust	0,280 [*]
Total impression, crumb	0,313 ^{**}
Evenness of slice surface	n.s.
Texture of crust	0,280 [*]
Texture of crumb	
firm/crumblly	n.s.
dry/doughy	n.s.
Flavour	0,352 ^{**}
Salt taste	0,409 ^{***}
Sweet taste	- 0,251 [*]

n.s. = non significant correlation

* - $p < 0,05$

** - $p < 0,01$

*** - $p < 0,001$

IV

Minerals and Phytate in the Analysis of Dietary Fiber from Cereals. I.

T. F. SCHWEIZER,¹ W. FRÖLICH,² S. DEL VEDOVO,¹ and R. BESSON³

ABSTRACT

Cereal Chem. 61(2):116-119

Considerable amounts of ash were found in dietary fiber in foods and especially in soluble-fiber components from cereal products in assays with an enzymatic gravimetric method. With wheat bran as a source of fiber, several digestion and isolation procedures were modified to identify the component minerals and the nature of their association with insoluble- and soluble-fiber components. When buffers with low ionic strength were used, citrate was substituted for phosphate, and soluble fibers were isolated with dialysis instead of precipitated with ethanol, as much as 90% of the ash in

the fiber fractions was reduced. On the other hand, more residual protein remained in the fiber fractions. From the assay of the quantitatively important minerals and of phytic acid in all fiber fractions and from separate solubility tests, coprecipitation was determined to be partly responsible for the ash in the soluble-fiber fractions. These results should be helpful in improving current analytical methods for determining dietary fiber.

Several approaches for analyzing dietary fiber in food are currently used, each having its own advantages and limitations (Southgate et al 1978, Schweizer 1980). When assaying the insoluble and soluble fibers by gravimetric methods after enzymatic digestion of protein and starch (Schweizer and Würsch 1979, 1981, Asp and Johansson 1981, Asp et al 1983), we found considerable amounts of ash, especially in the soluble-fiber fraction from cereals (Schweizer and Würsch 1979, Frölich and Asp 1981). Since no satisfactory explanation has yet been provided for this observation, studying the origin of this ash and the nature of its association to insoluble- and soluble-fiber fractions promised to be interesting.

The gravimetric method of Schweizer and Würsch (1979) for the assay of dietary fiber was used to perform blank determinations and digestions of wheat bran. Also, several modifications (type and ionic strength of buffer, and fiber-isolation method) were introduced to show to what extent the ash in the soluble-fiber fraction was caused by coprecipitation by the ethanol used for isolating soluble fibers, by the phytate associated with the soluble fibers, or by the ability of minerals to associate to the polysaccharides. Wheat bran was chosen as a fiber-rich material, because it is also rich in minerals and phytate. These three dietary components could be followed easily throughout the analytical procedures.

MATERIALS AND METHODS

Reagents and Samples

Analytical reagents of the purest grade available were used for all analyses. Other, specific products used are also mentioned.

Wheat bran was obtained from a local mill and ground to a particle size of less than 0.6 mm. The composition of the bran is given in Table I.

Solubility Tests

The following stock solutions were prepared:

1. 85 and 100 mM phosphate buffers, pH 6.0, by dissolving adequate amounts of $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ and $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ in distilled water;
2. 85 and 100 mM phosphate buffers, pH 4.5, by adjusting the pH of solution 1 to 4.5 with diluted HCl before adjusting the volume;
3. 85 and 100 mM citrate buffers, pH 6.0, by dissolving citric acid in distilled water and adding 25% ammonia solution until reaching pH 6.0 before adjusting the volume;
4. 85 and 100 mM citrate buffers, pH 4.5, by adjusting the pH of solution 3 to 4.5 with 5N HCl before adjusting the volume;
5. 333 mM sodium chloride;
6. 2.37 mM $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$;
7. 17.9 mM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$; and
8. 5.38 mM sodium phytate (Sigma Chemicals, St. Louis; % Na: 29.8 found, 29.9 calc; % P: 20.0 found, 20.1 calc).

The concentrations of the three latter salts were chosen to simulate, in the subsequent solubility tests, the enzymatic digestion of 2.5 g of wheat bran in a final volume of 150 ml, assuming the following amounts in wheat bran (mg/100 g): Ca, 114; Mg, 522; and

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phytic acid, 4,260. These are close to those found in food tables (Southgate and Paul 1978) and in the wheat bran used in this study.

The solubility tests were then performed by mixing the required amounts of the different solutions in a test tube to obtain the final concentrations given in Tables II-IV. Four volumes of ethanol were then added under stirring to the resulting aqueous solutions, and the tube contents were checked for turbidities and precipitates at room temperature after 10 min and after one night.

The results of Table IV were obtained by mixing 1 ml of one of the buffers 1-4, 0.5 ml of NaCl (5), 1 ml of the salts 6-8, and 0.5-2.5 ml of distilled water, followed by addition of 20 ml of ethanol in steps of 5 ml.

Dietary Fiber

The contents of insoluble and soluble fibers were determined as described by Schweizer and Würsch (1979, 1981), with some modifications. When citrate-buffer was used, the pH adjustment between the pepsin step and the pancreatin/glucosylase step was made with ammonia solution instead of NaOH. In some experiments, the pH was adjusted from 6.0 to 4.5 with HCl before the soluble fibers were isolated. As an alternative to solvent precipitation of the soluble fibers, these were recovered by exhaustively dialyzing the digestion filtrate against distilled water in Visking sacks, which, according to the manufacturer (Serva, Heidelberg, W. Germany), had an exclusion limit of 10,000 daltons, followed by freeze-drying of the retentate. The insoluble and soluble fibers were analyzed separately for residual nitrogen by the Kjeldahl method (conversion factor to protein = 6.25) and for ash.

The values reported in the tables are the means of two to four duplicate gravimetric analyses. The relative standard deviations were less than 3 and 5% for insoluble and soluble fibers, respectively, confirming the previously established reliability (Schweizer and Würsch 1979).

TABLE I
Composition of Wheat Bran (dry basis)

Total Composition	Percentage
Protein (N × 6.25)	16.9
Lipids	6.2
Starch	18.7
Sugars	4.2
Dietary fiber ^a	43.4
Ash	6.2
Phytic acid ^b	3.7 [4.4]
Ash components (mg/100 g)	
Na	7.4
K	1,500
Ca	122
Mg	470
P	1,250
Si	9.3

^aCorrected for residual protein and ash.

^bValues in brackets are calculated from P in ash, assuming that all P was from phytic acid.

TABLE II
Solubility of Buffers in 80% Ethanol (v/v) at Different pH^{a,b}

Concentration (mM)	Phosphate		Citrate	
	pH 4.5	pH 6.0	pH 4.5	pH 6.0
100	++	++	-	-
50	+	++	-	-
25	-	+	-	-
17	-	-	-	-
12.5	-	-	-	-

^aQualitative observations. - = entirely soluble, + = slightly cloudy, ++ = very cloudy.

^bConcentrations refer to the aqueous solutions, ie, before addition of four volumes of ethanol.

Minerals and Phytate

The samples were ashed to constant weight at 550°C and the resulting ashes dissolved in 2N hydrochloric acid. The individual cations were analyzed by flame atomic-absorption spectrophotometry. Silicon dioxide, although not soluble, was kept in homogenous suspension and could be determined by the same technique. The sum of the cations after conversion to their oxides accounted for 69-102% of the total ash. The complete recovery of phosphorus from the wheat bran and from sodium phytate was assessed in separate experiments by adding an excess magnesium oxide before ashing, as described in official AOAC methods 14.015 and 14.016 (Horwitz 1980).

The content of phytic acid in the bran and in the fiber fractions was determined by the method of Makower (1970) as modified by de Rham and Jost (1979).

RESULTS AND DISCUSSION

Solubility in Aqueous Ethanol of Buffer Salts, Minerals, and Phytic Acid

The concentrations of the phosphate buffers just before precipitating the soluble fibers in the gravimetric methods reported in the literature range from 16.7 mM at pH 6.0 (Schweizer and Würsch 1979) to 50 mM at pH 4.5.⁴ Therefore, the solubility of phosphate buffer salts around these concentrations was determined and compared to an ammonium-citrate buffer. The latter was

⁴N.-G. Asp, C.-G. Johansson, H. Hallmer, and M. Siljeström. 1983. A rapid enzymatic method for assay of insoluble and soluble dietary fiber. Presented at AOAC Spring Workshop, Ottawa.

TABLE III
Solubility of Buffer-NaCl Mixtures in 80% Ethanol (v/v)^{a,b}

Concentration of NaCl (mM)	Phosphate		Citrate	
	pH 4.5	pH 6.0	pH 4.5	pH 6.0
17 mM				
167	-	++	-	++
83	-	+	-	+
42	-	-	-	-
21	-	-	-	-
25 mM				
167	-	++	-	++
83	-	++	-	+
42	-	+	-	-
21	-	+	-	-

^aQualitative observations. - = entirely soluble, + = slightly cloudy, ++ = very cloudy.

^bConcentrations refer to the aqueous solutions, ie, before addition of four volumes of ethanol.

TABLE IV
Solubility in 80% Ethanol (v/v) of Minerals and Phytic Acid in 17 mM Buffer Containing 33 mM NaCl^{a,b}

Addition	Phosphate		Citrate	
	pH 4.5	pH 6.0	pH 4.5	pH 6.0
None	-	-	-	-
(A) Ca ²⁺ (19 µg/ml)	-	+	- ^c	-
(B) Mg ²⁺ (87 µg/ml)	+	++	-	-
(C) Phytic acid (710 µg/ml)	+	++ ^d	++ ^d	++ ^e
A + C	++ ^d	++ ^f	++ ^d	++ ^d
B + C	++ ^f	++ ^f	++ ^d	++ ^d
A + B + C	++ ^f	++ ^f	++ ^d	++ ^f

^aQualitative observations. - = entirely soluble, + = slightly cloudy, ++ = very cloudy.

^bConcentrations refer to the aqueous solutions, ie, before addition of four volumes of ethanol.

^cAfter prolonged standing only.

^dBeginning already at 67% EtOH.

^eBeginning already at 75% EtOH.

^fBeginning already at 50% EtOH.

chosen in view of the subsequent digestion experiments because it does not yield ash by itself.

Table II shows that the citrate buffer is soluble in 80% ethanol in a wider concentration range than phosphate. Addition of sodium chloride affects the solubility of the citrate more than that of the phosphate (Table III). This table also shows that much higher concentrations of sodium chloride can be tolerated at pH 4.5 than at pH 6. However, the sodium chloride produced in the procedure of Schweizer and Würsch (1979, 1981) through acidification in the pepsin step and subsequent neutralization does not generally exceed a final concentration of 30 mM. Therefore, no phosphate precipitation is expected, even if the sample analyzed contains up to 15% of sodium chloride. In the recently published procedure of Asp et al (1983), the final concentration of phosphate buffer was reduced to 25 mM at pH 4.5. Under these conditions, no precipitation is expected, despite the higher concentrations of sodium chloride obtained in this method.

Finally, Table IV shows the solubility of calcium, magnesium, and phytate in 80% ethanol. The conditions of this experiment were chosen to simulate the digestion of 2.5 g of wheat bran. The results indicate that, in contrast to the phosphates of calcium and magnesium, phytate can be expected to precipitate partially under all circumstances. Table IV also shows that the simultaneous presence of phytate, Ca, and Mg favors precipitation, since it occurs at lower concentrations of alcohol. On the other hand, these qualitative experiments cannot adequately simulate the more complex interactions between the minerals, phytic acid, and the polyonic fiber or protein components encountered in real digestion.

TABLE V
Composition of Insoluble Fibers from Wheat Bran (dry basis) Digested in Two Different Buffers^a

Composition of Insoluble Fibers	Phosphate	Citrate
Residue weight, %	42.9	43.0
Protein, % (N × 6.25)	3.9 (23.1)	4.9 (29.0)
Ash, %	0.8 (13.4)	0.2 (3.4)
Corrected residue weight, %	38.2	37.9
Phytic acid, %	0.19 (5.1) [0.34] ^b	0.03 (0.8) [0.09] ^b
Ash components (mg/100 g)		
Na	122 (1,650)	11 (149)
K	28 (1.9)	36 (2.4)
Ca	33 (27.2)	7 (5.7)
Mg	46 (9.8)	12 (2.6)
P	97 (7.8)	28 (2.2)
Si	10 (107)	11 (118)

^aValues in parentheses are the percentage of the content in the original bran.

^bValues in brackets are calculated from P in ash, assuming that all P was from phytic acid.

Determination in Wheat Bran of Dietary Fiber and Fiber-associated Ash

The content and composition of the insoluble fibers recovered from wheat bran by the method of Schweizer and Würsch (1979) are given in Table V. Residue weights and corrected residue weights were not greatly influenced by the choice of buffer, since the slightly lower residual protein content obtained with the phosphate buffer was compensated by the higher ash content. The average residual protein (26%) closely corresponds to the indigestible nitrogen fraction of wheat bran fed to rats (Saunders and Betschart 1980).

In both cases, silica was entirely recovered in the insoluble fibers. The ash, four times higher, obtained with the phosphate buffer was mainly due to sodium from the buffer that adhered to the fiber despite washing. Phosphorus, magnesium, and calcium were also higher, the latter accounting for 27% of the original calcium content of the bran. However, none of the minerals investigated remained adsorbed to the insoluble fibers to any important extent when citrate buffer was used. Only negligible amounts of phytate were recovered in the insoluble fibers with both buffers.

Table VI shows the corresponding data for soluble fibers. Residue weights and corrected residue weights were comparable for both buffers and all isolation methods, with the exception of the low amount of fibers precipitated from phosphate buffer at pH 4.5 with ethanol, for which we have no explanation. Replacing precipitation by dialysis in phosphate reduced the ash content of the fraction of the fiber from 40 to 10%, whereas the protein content increased from about 11 to 28% at both pH's studied. This confirms our previous findings (Schweizer and Würsch 1979). Phytic acid accounted for roughly 20% of the ash and for 50% of the phosphorus in the soluble fibers precipitated from phosphate buffer. Thus, phosphate from the buffer was coprecipitated, probably as magnesium phosphate (see Table IV). In all three alternative isolation procedures, phytic acid accounted for at least 80% of the phosphorus.

The results for the soluble fibers isolated from citrate buffer are also given in Table VI. As already shown for the insoluble fibers, the use of citrate leads to less ash in both isolation procedures. In contrast to the phosphate buffer, increasing the pH from 4.5 to 6.0 diminished calcium, magnesium, and phosphorus. This is in agreement with the qualitative data of Table IV.

Dialysis removed much of the ash in the soluble-fiber fractions, roughly 70 and 80% in phosphate and citrate, respectively. However, none of the minerals could be completely dialyzed, which suggested that coprecipitation might not be entirely responsible for the fiber-associated ash, and that some binding might nevertheless exist (Frølich and Asp 1981). This view is supported by the fact that separately prepared mixtures of phytate with magnesium or calcium are well dialyzed in comparable conditions.

Nevertheless, the apparent relationship between soluble fiber and the ash associated with it can now largely be explained by coprecipitation in 80% ethanol of phytate and related minerals. Thus, brans from different cereals (wheat, barley, rye, and corn)

TABLE VI
Composition of Soluble Fibers from Wheat Bran (dry basis), Recovered Under Different Conditions

Composition of Soluble Fibers	Percent Content in Bran	Phosphate Buffer				Citrate Buffer			
		80% Ethanol		Dialysis		80% Ethanol		Dialysis	
		pH 4.5	pH 6.0	pH 4.5	pH 6.0	pH 4.5	pH 6.0	pH 4.5	pH 6.0
Residue weight, %		7.3	9.6	9.7	10.1	9.9	8.8	9.3	8.3
Protein, % (N × 6.25)	5.3-16.6	0.9	1.0	2.8	2.8	1.8	2.3	2.3	2.6
Ash, %	4.8-61.3	2.9	3.8	1.0	1.0	2.3	1.4	0.5	0.3
Corrected residue weight, %	...	3.5	4.8	5.9	6.3	5.8	5.1	6.5	5.4
Phytic acid, % ^a	13.5-37.8	0.9 [1.8]	1.0 [1.9]	0.8 [1.0]	0.9 [1.1]	1.4 [1.4]	1.4 [1.3]	0.5 [0.6]	0.5 [0.4]
Ash components (mg/100 g)									
Na	122-6,311	196	467	153	105	16	20	13	9
K	1.2-13	140	193	29	22	139	110	18	35
Ca	7.4-107	95	99	12	28	131	53	18	9
Mg	4.7-89	319	417	54	85	179	59	52	22
P	8.6-42	510	526	290	322	397	373	172	107

^aValues in brackets are calculated from P in ash, assuming that all P was from phytic acid.

TABLE VII

Content of Soluble Fiber, Phytic Acid, and Soluble Fiber-associated Ash of Different Cereal Brans

Product	Soluble Fiber, % ^a	Ash, %	Phytic Acid, %
Wheat bran 1 ^b	4.8	3.8	3.7
Wheat bran 2 ^c	2.6	4.0	4.5
Rye bran ^c	2.6	2.5	2.5
Barley hulls ^c	1.1	1.4	0.6
Corn bran ^c	2.1	0.7	0.1

^a Corrected for residual protein and ash.^b Present study.^c Unpublished data (Schweizer 1980).

with widely different phytic acid contents show a close relationship ($r = 0.99$) between their phytate content and their fiber-associated ash (Table VII). The relationship between soluble fiber and fiber-associated ash ($r = 0.69$) is clearly less pronounced.

CONCLUSION

Our findings show that the majority of Ca and Mg is released from wheat bran by the enzymatic treatment, which is similar to the one in the upper gastrointestinal tract. This release can be assumed to be largely due to the acidic pepsin-digestion step (Thompson and Weber 1979). Upon neutralization and digestion with pancreatin, there is little binding of the minerals to the insoluble fibers, especially when the chelating citrate buffer is used. The apparent association of the minerals with the soluble fibers is largely due to their coprecipitation in aqueous ethanol, since the majority of the minerals is removed from the digest when this isolation procedure is replaced by dialysis.

The results of this study provide some new information regarding current analytical practice. Because the majority of ash and protein in the soluble fibers originate from coprecipitation with 80% ethanol, it might be adequate to correct the soluble fiber fraction for residual protein and ash. The pure gravimetric value, including the indigestible protein, can be used for the insoluble fibers (Schweizer and Würsch 1981). If, however, total dietary fiber is determined gravimetrically, without separating insoluble and

soluble fibers, the convention of correcting for residual ash and protein could be adopted. The buffer strength used should be kept at a low level, however, to minimize coprecipitation and the corrections for residual ash. The alternative use of citrate-buffer or dialysis merits further attention, but the substantial increase of residual protein in the soluble fibers calls for more efficient proteolytic steps, before such an alternative can be recommended.

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[Received July 5, 1983. Accepted October 11, 1983]

V

Minerals and Phytate in the Analysis of Dietary Fiber from Cereals. II

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ABSTRACT

Cereal Chem. 61(4): 357-359

Dietary fiber and especially soluble fiber components in a wheat bread dough with a high content of fiber were associated with considerable amounts of ash when assayed with an enzymatic gravimetric method, using ethanol precipitation to recover soluble fiber. Only a small amount of minerals occurred in the insoluble fiber fraction, except for iron, 40% of which was associated with this fraction. Only a fraction of the minerals and phytate associated with the alcohol-precipitated soluble fiber could be removed by dialyzing the redissolved fiber. Soluble fiber dialyzed without

previous alcohol precipitation contained much smaller amounts of minerals, but nearly 30% of the iron was bound to the soluble fraction also when prepared without ethanol precipitation. This study shows that the isolation procedure of fiber components heavily modifies their mineral-binding capacity. However, the different cations investigated (Ca, Mg, Fe, and Zn) were not uniformly affected by the isolation procedures. Great care is needed when extrapolating from *in vitro* studies with isolated fiber components to the native dietary fiber or to its effect *in vivo*.

Previous studies (Schweizer and Würsch 1979, Frølich and Asp 1980) demonstrated that a considerable amount of ash was associated with dietary fiber assayed with enzymatic, gravimetric methods. This ash was found almost exclusively in the soluble-fiber fraction, but the nature of this association has not yet been elucidated.

Some observations about the influence of buffer choice, ionic strength, and fiber-isolation method on fiber-associated minerals were reported in part I of this study (Schweizer et al 1984), in which bran was the fiber source. For this second part of the study, a bread dough was chosen as the fiber source, and more minerals were included. In addition to studying methodology, the purpose of the study was to contribute to the knowledge of *in-vitro* mineral binding to cereal fiber.

MATERIALS AND METHODS

Samples

Four bread doughs were prepared from whole grain wheat flour with an addition of bran. The dough recipe was as follows: water, 700 ml; whole grain wheat flour; 700 g; bran, 300 g; salt, 17.5 g; and yeast, 35 g. Samples were taken for analysis directly after the ingredients were mixed.

Sample Preparation

The samples did not contain more than 2–3% lipids and were therefore not defatted (Schweizer and Würsch 1979, Asp et al 1983). The samples were immediately dried at 105°C for 2 hr and ground in a Cyclotec sample mill (Tecator AB, Höganäs, Sweden) to a particle size of <0.45 mm. Ash content was determined by ashing at 500°C to constant weight.

Mineral Determination

After ashing, the samples were dissolved in a 1:1 solution of 2% (v/v) HCl and HNO₃. The individual minerals were then determined by plasma spectrophotometry by emission, using a Direct Reading Spectrometer (Simultaneous Instrument, no. 975 I CAP, Jarrell-Ash, Boston).

Dietary Fiber

The method used to determine dietary fiber content was based on

Hellendoorn's enzymatic, gravimetric method (Hellendoorn et al 1975) as described by Asp et al (1983).⁴ To remove starch completely, an extremely heat-stable α -amylase (Termamyl 60L, Novo, Copenhagen, Denmark) was used in an initial gelatinization step at 100°C for 15 min. Further enzyme digestions were performed with pepsin (Merck no. 7190) at pH 1.5 for 1 hr, and with pancreatin (Sigma no. P-1750) at pH 6.8 for 1 hr, both at 40°C. The mineral content (mg) of the enzyme preparations used for a 1-g sample (dry matter) of bread dough was as follows: Termamyl (100 μ l): Ca 0.46, Mg 0.06, P 0.20, Fe 0.001, Zn 0.001; pepsin (100 mg): Ca 0.05, Mg 0.14, P 0.58, Fe 0.003, Zn 0.002; pancreatin (100 mg): Ca 1.50, Mg 0.06, P 1.00, Fe 0.007, and Zn 0.014.

The final phosphate buffer concentration used in this study before ethanol precipitation was 50 mM. In view of the present results, the phosphate buffer concentration was decreased to 25 mM in the final version of the method as described by Asp et al (1983).

The fiber components were recovered either by filtration or by centrifugation. In filtration, insoluble-fiber components were recovered by filtration and soluble components by precipitation with four volumes of 95% ethanol (final concentration, 78%; v/v), followed by filtration. The filtrations were done with Tecator's Fibertec system (Tecator AB, Höganäs, Sweden), using 0.5 g of Celite 545 as a filter aid. In centrifugation, insoluble-fiber components were recovered by centrifugation for 30 min at 1,600 $\times$ g in a Beckman centrifuge J 2-21 at 4°C. The supernatants and washings (100 ml) were precipitated with four volumes of ethanol, and the soluble-fiber components were recovered by centrifugation in the same centrifuge for 30 min at 3,000 $\times$ g at 4°C.

The dietary fiber values reported in the tables are means of duplicate gravimetric analyses. All values are given in percent, corrected for protein (Kjeldahl N $\times$ 6.25) and ash associated with the fiber.

Phytate

Phytate was determined with a modification of Holt's method (Holt 1955). This method is based on complex formation of phytic acid and Fe(III)-ions at pH 1–2. An excess of Fe(III) present in the solution will react with thiocyanate ions to form a characteristic pink complex, Fe(SCN)₃. The optical density at 465 nm in an amyl-alcohol layer is measured, and an inverse linear relation is found for phytate concentrations ranging from 40 to 200 nmol/L.

Dialysis

The alcohol-precipitated fiber fractions were redissolved in 2 ml of distilled water. The solutions were dialyzed against 4 L of double-distilled water for 48 hr at 4°C in Spectrapor sacks (Spectrum Medical Industries, Inc., Los Angeles, CA) with an exclusion limit of 6,000–8,000 daltons. The contents of the sack were then freeze-dried.

As an alternative to the method described above, the soluble

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⁴N. G. Asp., C.-G. Johansson, H. Hallmer., and M. Siljeström. 1981. A rapid enzymatic method for assay of insoluble and soluble dietary fiber. Presented at AOAC Spring Workshop, Ottawa.

fiber was recovered by dialyzing the supernatant obtained after centrifuging the enzyme digest, using the same procedure as above. In this experiment with direct dialyzing, the Termamyl was excluded.

RESULTS AND DISCUSSION

Table I gives dietary fiber content of the bread dough, with both the filtration and the centrifugation methods. The total dietary fiber values (corrected for ash and undigestible protein) were the same with the two procedures. However, the soluble fraction was significantly larger with the centrifugation method. The amount of ash associated with soluble fiber was the same with both methods, whereas slightly more ash was found in the insoluble fiber when the filtration method was used. The reason for recovering the fiber components with centrifugation, which is a more time-consuming method, was that the filtration aid, Celite, contains minerals that disturb the assay of individual minerals.

Table II gives the composition of the bread dough with regard to dietary fiber, phytate, ash, and selected minerals. About 70% of the phosphorus in the bread dough is present as phytate.

Individual mineral determinations (Tables III and IV) show similar distribution between insoluble and soluble fiber as total ash (Table I). Only 1.6–13.2% of the total minerals were associated with

insoluble fiber. The only exception was iron, 40% of which was associated with this fraction. For some minerals (Ca and Zn), the amount recovered in the soluble-fiber fraction exceeded that in the dough sample analyzed. This can be explained by the mineral content of the enzyme preparations given in Materials and Methods. Termamyl and pancreatin contained large amounts of calcium, and pancreatin also contained a significant amount of zinc.

TABLE II
Composition of Two Samples of Wheat Bread Dough (db)

Components	Percent	Mean Range (mg/g)
Dietary fiber ^a	24.4 ± 1.1	
Phytate	1.7 (2.4) ^b	
Total ash	3.0	
Ca		0.59; 0.59
Mg		2.61; 2.55–2.68
P		6.80; 6.70–6.90
Fe		0.083; 0.082–0.084
Zn		0.053; 0.053–0.054

^a Corrected for residual protein ash.

^b Values in parentheses are calculated from P in ash, assuming that all P was from phytate.

TABLE III
Composition of Components of Insoluble Fiber in Four Samples of Bread Dough (db)

Components	Percent	Mean (mg/g) ^b	Range (mg/g)
Residue weight	23.5 ± 0.9		
Ash	0.5 (16) ^a		
Phytate	Not detectable		
Insoluble fiber ^a	20.6 ± 0.9		
Ca		0.077	0.075–0.090 (13.1)
Mg		0.044	0.035–0.055 (1.6)
P		0.47	0.27–0.69 (6.8)
Fe		0.031	0.027–0.034 (37.3)
Zn		0.007	0.006–0.009 (13.2)

^a Corrected for residual protein ash.

^b Values in parentheses are percentage of total content in the dough.

TABLE I
Content of Dietary Fiber in the Bread Dough Determined with Centrifugation/Filtration^a

	Centrifugation Method	Filtration Method
Dietary fiber (corrected for residual protein and ash)		
Insoluble fiber	20.7 ± 0.9	21.3 ± 1.8
Soluble fiber	3.7 ± 0.8	2.7 ± 0.3
Total fiber	24.4 ± 1.1	24.0 ± 2.1
Ash associated with dietary fiber		
Insoluble fiber	0.2	0.4 ± 0.2
Soluble fiber	2.4 ± 0.3	2.5 ± 0.5

^a All figures in percent of dry matter of the dough. Means ± SD of duplicate determinations for each of four doughs. Statistical evaluation was made with Student's *t*-test.

TABLE IV
Composition of Soluble Fiber in Four Samples of Bread Dough (db)

Components	Percent	Mean (mg/g)	Range (mg/g)
Residue weight	6.5 ± 0.8		
Ash	2.4 ± 0.3 (80) ^a		
Phytate	0.8 (46.5) [2.2] ^b		
Soluble fiber ^c	3.7 ± 0.8		
Minerals associated with soluble fiber recovered by ethanol precipitation			
Ca		1.60	1.25–1.95 (270)
Mg		1.70	1.65–1.75 (65)
P		6.10	5.30–6.90 (90)
Fe		0.063	0.058–0.071 (76)
Zn		0.091	0.071–0.117 (170)
Dialysis of redissolved ethanol-precipitated fiber			
Ca		1.40	1.00–1.65 (230)
Mg		1.35	1.30–1.40 (52)
P		3.40	2.90–3.80 (50)
Fe		0.048	0.043–0.054 (59)
Zn		0.074	0.065–0.086 (140)
Dialysis of supernatant without ethanol and Termamyl			
Ca			0.13 (22)
Mg			0.037 (1.4)
P			0.840 (12)
Fe			0.023 (28)
Zn			0.006 (11)

^a Values of parentheses are percentage of total content in the bread dough.

^b Values in parentheses are calculated from P in ash, assuming that all P was from phytate.

^c Corrected for protein and ash.

High amounts of phosphorus were also associated with the fiber, which could be partly due to coprecipitated phosphorus from the 50 mM phosphate buffer. Similar findings were reported for bran in part I of this work (Schweizer et al 1983), although the amount of phosphorus was somewhat lower, because of the lower phosphate concentration (17 mM) used in that study.

Nearly half (47%) of the content of phytate in the original bread dough was recovered in the soluble-fiber fraction, whereas no phytate could be detected in the insoluble-fiber fraction. The supernatant after precipitation and filtration of the soluble fiber contained 35% of the original phytate. The last 15% could possibly have been broken down during the enzyme incubation steps in the fiber-assay procedure.

In an attempt to measure whether the minerals were really bound to the fiber or just coprecipitated through the ethanol, two experiments were set up. In one experiment, the precipitated soluble fibers were redissolved and dialyzed against water. The redissolution was performed both in distilled water and in hydrochloric acid (pH 4.5), with the same result. Surprisingly, only about 20% of the minerals were dialyzable, except for phosphorus, 40% of which disappeared. In the other experiment, the supernatant was dialyzed directly after the centrifugation of the enzyme digest. Most of the minerals could then be dialyzed (Table IV). In this experiment, Termamyl was omitted in the digestion to avoid unnecessary mineral addition. This gives approximately 1% residual starch in the fiber residue (Asp et al 1983). By replacing precipitation by dialysis, the total ash associated with the soluble-fiber fraction decreased to about one-fourth. All of the magnesium and nearly all of the zinc and phosphorus disappeared through dialysis. In contrast, 28% of the iron in the bread dough remained bound to the soluble fiber after this procedure. These results are in agreement with the findings with bran, where most of the minerals could be dialyzed away from the fiber (Schweizer et al 1983). Therefore, it seems that the soluble fiber or the phytate is altered through ethanol precipitation in such a way that minerals are not released upon redissolution. This clearly shows that the procedure used for isolation of dietary fiber can heavily modify its ion-binding properties. Therefore, great care is needed when extrapolating from in-vitro studies with fiber fractions treated with solvents or detergents to the original fiber sources (Fernandez and Phillips 1982). This has also been pointed out with respect to the bile-acid binding of fibers (Story et al 1982).

CONCLUSION

This study shows that the mineral binding of dietary fiber fractions in-vitro is modified by solvent precipitation. Studies done on isolated fiber fractions do not directly reflect native dietary fiber, and such studies cannot be extrapolated to the effect of dietary fiber in human beings.

The majority of the ash and nearly half of the phytate were associated with the soluble-fiber fraction after isolation of the latter by solvent precipitation. The insoluble-fiber fractions did not contain measurable amounts of phytic acid and contained only minor amounts of minerals. As an exception to this, 37% of the iron in the dough remained associated with this fraction. Iron associated with the soluble fraction also seems to behave differently than other minerals. When the soluble fiber fraction was dialyzed without being alcohol precipitated and redissolved, 28% of the iron remained bound to this fraction, whereas around 90% of other minerals were removed.

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[Received September 6, 1983. Revision received January 13, 1984. Accepted February 16, 1984]

VI

MINERALS AND PHYTATE IN THE ANALYSIS
OF DIETARY FIBER FROM CEREALS
DURING THE PROCESS OF BREADMAKING
PART III

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ABSTRACT

A breaddough with a dietary fiber content of 24% of dry matter was fermented up to 17 hours and baked. Changes in content of dietary fiber and phytate, and mineral association to these components in vitro were measured during the processes.

The total dietary fiber content was not influenced by fermentation or baking, even though a slight tendency to loss of soluble fiber components and increase of insoluble fiber components could be observed.

Ash was associated mainly to the soluble fiber fraction. During fermentation and breadmaking there was a decrease in fiber associated ash.

Even with extremely long fermentation it was impossible to obtain complete hydrolysis of phytate. With 17 hours of fermentation it was reduced only to half of that present in the dough at zero time.

Nearly three quarter of the phytate was detectable in the soluble fiber fraction, and no phytate in the insoluble fiber fraction.

By specific mineral analysis it was shown that different minerals behave differently, both concerning binding to fiber fractions before fermentation and behavior during fermentation.

Up to 60% of the total iron was associated to other components in the dietary fiber complex than phytate. Only 10% of the total iron seemed to be associated to phytate.

Zinc and calcium, 24% and 60% respectively, seemed to be associated with the phytate in the soluble fiber fraction, and were released during fermentation. 9% of the magnesium was also found associated to this fraction and released during fermentation.

All phosphorus in the soluble fiber fraction seemed to be present as phytate.

KEY WORDS

Cereals, fermentation, baking, dietary fiber, phytate, minerals.

INTRODUCTION

The content of minerals, dietary fiber and phytate increase with increasing extraction rate of the flour. Phytate is known to have chelating properties and to form complexes with divalent cations. Also other factors in the dietary fiber complex have been pointed out as possible chelators. (Reinhold et al 1976a, 1976b, Ismail-Beigi 1977a and 1977b). It has been shown by Reinhold et al (1981) that dephytinized wheat bran bound iron as effectively as native bran. Bread fermented for 15 hours gave an increased zinc absorption compared with bread with no fermentation. (Nävert et al 1984).

In previous papers we have shown (Schweizer et al 1984, Frølich et al 1984) that considerable amounts of ash were associated with dietary fiber components, mainly to the soluble fiber fraction, where also most of the phytate was recovered. Only a small fraction of minerals occurred in the insoluble fractions, except for iron, up to 40% of which was recovered in this fraction. No phytate could be detected in this fraction.

Preparation of leavened bread with yeast fermentation step allows yeast and wheat phytases to hydrolyse phytic acid. Ranhotra et al (1974) found that all of the phytate in wheat bread was hydrolysed during the process of bread baking, which is in contrast to the study of Harland and Harland (1980). The present study investigates the association of minerals to the different fiber fractions in vitro during the process of bread baking, both fermentation and baking. This could give an indication to which components in the dietary fiber the minerals were associated, and how this association was influenced by fermentation and baking procedure. It was also of interest to see if there were any changes in dietary fiber components during these processes.

MATERIALS AND METHODS

Samples

Breaddoughs were made from whole grain wheat flour with an addition of bran. The recipe of the doughs was as follows: Water 700 ml, whole grain wheat flour 700 g, wheat bran 300 g, salt 17.5 g and yeast 35 g.

The dough was fermentated 2-17 hrs and then baked for 3/4 of an hour.

Samples were taken at intermediary times during bread making; at zero time (directly after mixing the ingredients): A_1 , after 2 hrs fermentation: A_2 , after 8 hrs fermentation: A_3 , after 17 hrs fermentation: A_4 , and after 17 hrs fermentation with an additional baking at 3/4 of an hour: A_5 .

Sample preparation

All samples were immediately dried in a microwave oven with an additional hour at 105°C in a traditional oven. The samples were ground in a Cyclotec sample mill (Tecator AB, Höganäs, Sweden) to particle size less than 0.45 mm and kept in closed containers.

All samples were analyzed for phytic acid, insoluble and soluble dietary fiber, ash and specific mineral content. Ash content was determined by ashing at 500°C to constant weight.

Mineral determination

After ashing, the samples were dissolved in a 1:1 solution of hydrochloric and citric acid, final concentration of 4% v/v of both acids. The in-

dividual minerals were then determined by plasma spectrophotometry by emission, using a "Direct reading spectrometer", Simultaneous Instrument, No 975 I CAP (Manufacturer Jarrell - Ash).

Dietary fiber

The method used to determine dietary fiber content was based on Hellendoorn's enzymatic, gravimetric method (Hellendoorn et al 1975) as described by Asp et al (1981, 1983): To remove starch completely, an extremely heat-stable α -amylase (Termamyl 60L, Novo, Copenhagen, Denmark) was used in an initial gelatinization step at 100°C for 15 min. Further enzyme digestions were performed with pepsin (Merck no 7190) at pH 1.5 for 1 hr and with pancreatin (Sigma no P-1750) at pH 6.8 for 1 hr at 40°C. The mineral content of the enzyme preparations used for a 1 g sample (dry matter) of bread dough was as follows (mg): Termamyl (100 μ l); Ca 0.46, Mg 0.06, P 0.20, Fe 0.001, Zn 0.001. Pepsin (100 mg): Ca 0.05, Mg 0.14, P 0.58, Fe 0.003, Zn 0.002, Pancreatin (100 mg); Ca 1.50, Mg 0.06, P 1.00, Fe 0.007, Zn 0.014.

Two kinds of buffer system were used in the studies of mineral association, phosphate buffer and citrate buffer. The final buffer concentrations before ethanol precipitation were 25 mM for both buffer system, to get as low coprecipitation of phosphorus and other minerals as possible. (Schweizer et al 1984, Frølich et al 1984). For the same reason the Termamyl step was omitted in these studies.

The fiber components were recovered either by a) filtration (determination of dietary fiber content) or b) centrifugation (studies of mineral association).

a) Insoluble fiber components were recovered by filtration and soluble components by precipitation with four volumes of 95% ethanol (final concentration 78% v/v) followed by filtration, using phosphate buffer in the assay. The filtration was carried out with Tecator's Fibertec system. (Tecator AB, Höganäs, Sweden) using 0.5 g Celite 545 as a filter aid.

b) Insoluble fiber components were recovered by centrifugation for 30 min at 1600 x G in a Beckman centrifuge J2-21 at 4°C. The supernatants and washings (100 ml) were precipitated with 4 volumes of ethanol and the soluble fiber components were recovered by centrifugation in the same centrifuge for 30 min at 3000 x G at 4°C. In the fiber assay with centrifugation, citrate buffer was used, to avoid coprecipitation of phosphate.

The dietary fiber values reported in the tables are means of duplicate gravimetric analyses. Values are given in percent of dry matter, if nothing else is indicated, corrected for protein (Kjeldahl N x 6.25) and ash associated with the fiber.

Dialysis

In some experiments the soluble fiber was recovered by dialyzing the supernatant obtained after centrifuging the enzyme digest. The solutions were dialyzed against 4 liters double distilled water for 48 hrs at 4°C in Spectrapor sacks (Spectrum Medical Industries, Inc., Los Angeles, USA) with an exclusion limit of 6000-8000 daltons. The sack content was lyophilized. In this experiment Termamyl was excluded in the assay, due to the rather high mineral contents of this enzyme preparation.

The dialysis procedure to obtain soluble fiber was used to avoid possible structural alteration of soluble fiber or phytate obtained by precipitation with alcohol followed by redissolution before dialyzing which has been pointed out in a previous study (Frølich et al 1984).

Phytate

Insoluble and soluble fiber components of the five samples recovered by centrifugation, were analyzed for phytate.

Phytate was determined with Holt's method (Holt 1955). This method is based on complex formation of phytic acid and Fe (III)-ions at pH 1-2. Excess of Fe (III) present in the solution will react with thiocyanate ions to form a characteristic pink complex, $\text{Fe}(\text{SCN})_3$. The OD at 465 nm in an amylalcohol layer is measured and an inverse linear relation is found for phytate concentrations ranging from 40-200 nmoles/l.

Phytase preparation

The enzyme phytase was prepared from wheat according to the method of Peers (Peers et al 1953) to get an enzyme with optimal activity. The enzyme was kept in a freezer (-20°C).

Phytase incubations

Some of the supernatants obtained after the first centrifugation of the enzyme digest, containing the soluble fiber components, was incubated for 18 hrs with phytase at 37°C . The enzyme reaction was stopped with 0.1 M TCA and neutralized back to pH 4.5 with NaOH. The incubate was analyzed for phytate and dialyzed. The content of the dialyzing sacks were analyzed for phytate and specific minerals after the dialysis.

RESULTS AND DISCUSSION

Dietary fiber content in dough and bread

Table 1 gives dietary fiber content of the five different bread doughs. The total dietary fiber content was not influenced by fermentation or baking. It seems, though, to be a slight tendency to loss of soluble fiber components. A slight tendency to increase of insoluble fiber components was also seen, which could be explained by formation of resistant starch (Johansson et al 1984). It has also been pointed out (Theander 1983) that the content of lignin might be increased with increasing temperature.

Protein and ash associated with dietary fiber

The main part of protein associated with the dietary fiber occurred in the insoluble fraction. During fermentation and breadmaking the amount of associated protein seemed to be constant both for insoluble and soluble fiber components. The ash associated to dietary fiber on the other hand occurred mainly in the soluble fiber fraction, which has been pointed out in previous papers (Frølich et Asp 1980, Frølich et al 1984). There was a decrease in this ash with increasing fermentation times, which was true also for individual minerals (Table 3). Baking of bread seems to reassociate some of the minerals. This is shown both for total ash and individual minerals.

Fate of phytate

Table 2 shows the fate of phytate during the fermentation and baking process, both in the whole bread dough and in different fiber fractions.

After 17 hrs of fermentation the phytate was reduced to about half of that present in the dough at zero time. Even with extremely long fermentation it was impossible to obtain complete hydrolysis of phytate, which is in accordance with the findings of Harland and Harland (1980).

During the baking process an additional breakdown of phytate to about 40% of the original amount occurred.

Before the fermentation nearly three quarter of the phytate was detectable in the soluble fiber fraction. The rest of the phytate present in the bread dough was recovered in the supernatant obtained after ethanol precipitation and centrifugation.

During fermentation the phytate associated to soluble fiber components was broken down, more or less to the same extent as the phytate in the dough. The phytate content in the supernatant after alcohol precipitation on the other hand was constant, regardless of fermentation period or baking.

When dialysis was used to recover soluble fiber components, approximately the same amount of phytate was found associated with this fraction as when recovered by ethanol precipitation and centrifugation.

These findings could be explained in two ways:

- 1) The main part of phytate is associated to soluble fiber components and will be coprecipitated with ethanol together with these components.

When the soluble fiber components are recovered with dialysis, the phytate will remain together with the soluble fiber components in the sack after dialyzing.

2) The phytate molecules would to a great extent, be complexed in aggregates, which will be precipitated together with the soluble fiber components during ethanol precipitation. These phytate aggregates are too big to pass the dialysis sack used in the dialysis experiment.

Some of the phytate molecules on the other hand are not associated to soluble fiber components and/or are small enough to pass through the sack used during dialysis and will not be precipitated with ethanol during the precipitation step.

A further reduction of the phytate could be observed with an additional incubation with phytase prepared from wheat. The supernatant from the first centrifugation after enzyme digestion in the fiber assay (including soluble fiber) was incubated for additional 18 hrs at 37°C. But even with such an extremely long incubation time, a complete hydrolysis of phytate was not obtained.

Most probably, the most active phytase in the dough, is the phytase from yeast. It has been shown by Harland and Harland (1980) that the hydrolysis of phytate could be increased by increasing the amount of yeast in the dough. In our study the phytase added for the incubation experiment, is on the other hand, prepared from wheat. The phytate available for this specific phytase, might be less and more limited, than the phytate available for the phytase from yeast.

Fate of specific minerals

The content of selected, specific minerals in the bread dough is shown in Table 3. The mineral contents of the same minerals associated to insoluble and soluble fiber components are also shown in the same table.

Most of the minerals associated to fiber components occur in the soluble fiber fraction, which has been pointed out in previous papers (Frølich et Asp 1980, Schweizer et al 1984, Frølich et al 1984). The minerals, though, behave differently, both concerning binding to fiber fractions before fermentation and behavior during fermentation.

Although the amount of ash associated to insoluble fiber components was small and not influenced by fermentation, 35-40% of the iron present in the bread dough occurred in this fraction. 23% of the calcium and 16% of the zinc, but only 3-4% of the magnesium and phosphorus were also found in the insoluble fiber fraction. However, no phytate could be detected, which is in accordance to the low amount of phosphorus.

The picture for the minerals associated to soluble fiber components was different. 61% of calcium, 29% of iron, 24% of zinc and phosphorus, but only 9% of magnesium in the bread dough was recovered in this fraction. During fermentation there was a decrease in minerals associated to this fraction. The content of soluble fiber components during this process was only slightly changed, while a decrease in the phytate was observed. Thus, much of the mineral in the soluble fiber fraction seems to be complexed with phytate and liberated during hydrolysis of phytate. All the phosphorus recovered in the soluble fiber fraction seems to be present as phytate. (0.65% phytate of the bread dough at zero time and 0.20% after 17 hrs fermentation).

Iron

As mentioned earlier considerably more of the total iron was associated to the insoluble fraction, compared with the other minerals. The iron associated to soluble fiber fraction also behaved differently than the other minerals in relation to phytate degradation.

The amount of iron associated to this fraction only decreased the first two hours of fermentation, from 30 to 20% of the total iron in the breaddough, and was constant at around 20% after that.

Even with the additional 18 hrs of incubation with phytase, this amount was constant. This could indicate that only 10% of the iron is associated to phytate, while up to 60% is associated to fiber components, both insoluble and soluble, not being affected by fermentation.

During fermentation there was an elimination of available binding sites due to the hydrolysis of phytate. A complete hydrolysis of phytate was never obtained. However, it has been claimed by Brown et al (1961) that only eight of the twelve dissociated protons of phytate are available for metal binding. Most probably, the remaining phytate does not have the ability to bind minerals. This is supported by the fact that zinc which is known to associate strongly with phytate, was not associated to the remaining phytate after incubation and dialysis. The same was true for magnesium.

This is in contrast to previous reports of iron, where it is claimed that it is associated mainly to phytate (Morris et al 1976) and being released during fermentation.

Zinc, magnesium, calcium

Zinc and magnesium seemed to be associated with phytate, as they are released from the soluble fiber fraction during fermentation. With the additional phytase incubation both minerals are totally released. The calcium present in the soluble fiber fraction was decreased from 60% to 25% during the 17 hrs of fermentation. With additional incubation calcium was further released to under 10% of the total calcium present in the original breaddough.

Calcium seems to be associated tighter to the phytate than e.g zinc and magnesium. It has been suggested that there is a complexation between calcium, magnesium and zinc to the phytate molecule (Likuski 1965, Oberleas et al 1966a, 1966b, Lease 1967). An explanation for our findings could be that magnesium and zinc are easier released from this complex than calcium.

In the soluble fiber fraction a small amount of calcium is not released during fermentation. It is recognized that calcium is bound not only to phytate, but also to fiber components, as pectin and other non-cellulosic fiber. This could be the explanation for the remaining calcium in the soluble fiber fraction. (James 1980).

A reassociation of some of the minerals seems to occur during the breadmaking. This could be due to Maillard reaction products. (Johnson et al 1983).

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TABLE I
CONTENT OF DIETARY FIBER
IN BREADDOUGH AFTER DIFFERENT
FERMENTATION PERIODS

(All figures in percent of dry matter in the dough,
corrected for residual protein and ash)

Samples				Protein	Protein	Ash	Ash
				associated	associated	associated	associated
				to	to	to	to
				insoluble	soluble	insoluble	soluble
	Insoluble	Soluble	Total	fiber	fiber	fiber	fiber
	fiber	fiber	dietary	fraction	fraction	fraction	fraction
			fiber				
A ₁	21.3	2.4	23.7	3.8	1.5	≤0.2	3.1
A ₂	21.8	2.5	24.3	4.7	1.8	≤0.2	2.5
A ₃	21.7	2.4	24.1	4.1	2.0	≤0.2	2.5
A ₄	22.2	2.1	24.3	2.9	1.5	≤0.2	1.8
A ₅	22.4	1.5	23.9	3.3	1.5	≤0.2	3.3

TABLE 2
FATE OF PHYTATE DURING FERMENTATION AND BREADMAKING
(mg/g dry matter of the breaddough)

Samples	Phytate in breaddough	Phytate insoluble in fiber fraction	Phytate in the soluble fiber fraction, recovered by ethanol precipitation			Phytate in soluble fiber fraction, after incubation 18 hrs with phytase and dialysis
			Phytate in the soluble fiber fraction, recovered by ethanol preci- pitation	Phytate in supernatant, recovered after precipitation and centrifugation	Phytate in soluble fiber fraction, recovered by dialysis	
A ₁	17.9	not detectable	13.8	5.9	11.7	7.9
A ₂	16.4	"	9.3	5.3	9.9	6.3
A ₃	11.4	"	4.2	5.3	5.3	3.0
A ₄	9.0	"	4.2	5.5	2.2	1.0
A ₅	7.2	"	2.0	6.6	2.9	1.0

% phytate in the dough : 1.8%

% phytate if all P in the dough were phytate 2.7%

% P as phytate : 65%

TABLE 3

CONTENT OF MINERALS IN THE BREADDOUGH AND IN THE DIFFERENT FIBER FRACTIONS AFTER FERMENTATION AND BREADMAKING, AND INCUBATION OF THE SOLUBLE FIBERS

Minerals in the breaddough mg/g dry breaddough

Ca	Mg	P	Fe	Zn
0.59	2.80	7.20	0.081	0.055

Minerals associated to different fiber fractions (% of minerals present in dry breaddough)

Samples	Associated to insoluble fiber					Associated to soluble fiber (recovered by dialysis)					Associated to soluble fiber after additional 18 hrs incubation with phytase of the soluble fractions. (recovered by dialysis)				
	Ca	Mg	P	Fe	Zn	Ca	Mg	P	Fe	Zn	Ca	Mg	P	Fe	Zn
A ₁	23	3	4	35	16	61	9	24	29	24	7	0.4	4	16	5
A ₂	22	3	4	35	16	42	6	17	19	16	6	0.2	5	16	0
A ₃	23	3	3	32	13	29	6	14	21	7	9	0.5	6	17	0
A ₄	23	3	4	43	18	25	2	9	19	14	8	0.4	6	21	0
A ₅	24	3	4	33	14	24	5	17	21	7	16	0.8	7	16	0

VII

Bioavailability of iron from wheat bran in pigs^{1, 2}

Wenche Frølich and Arvid Lysø

ABSTRACT Iron anemia was induced in pigs immediately after birth by feeding an iron depletion diet containing only 17 mg iron/kg feed. (The requirement for iron in this period is 50 mg iron/kg feed.) When Hb concentrations were 5 g/100 ml the pigs were given iron repletion diets. One group received 7% bran in the diet, about 60% of the iron derived from the bran and 40% from ferrous sulfate. The other group received no bran and 80% of the iron from ferrous sulfate. There were no differences, either in the increase of Hb or in the increase of serum iron, in the two groups. In a second experiment, one group received all their iron from cereals, and an addition of 20% bran in the diet. The other group received no bran and 80% of the iron from ferrous sulfate. There was no significant difference in the bioavailability of the iron present in the diets. In our experiments bran seemed to have no inhibitory effect on iron absorption, even when 20% bran was included in the diet. *Am J Clin Nutr* 1983;37:31-36.

KEY WORDS Iron, bioavailability, wheat bran, pigs

Introduction

Whole wheat flour and wheat bran are both products with a high content of dietary fiber (1). Both dietary fiber and factors associated with it, eg, phytate, have raised much concern with regard to nutrition. These factors may interfere with and reduce the bioavailability of iron, as indicated by several authors (2-5). The effect of phytate in wheat bran on iron absorption is debatable. Other factors in whole grain bread, eg, dietary fiber, may be chiefly responsible for the poor bioavailability of iron from wheat with high extraction rats. Some reports, however, have stated the absorption of native iron from both bread and bran to be greater than that from added inorganic iron (6, 7). It has also been stated that iron absorption does not appear to be affected by dietary fiber (8). It may be due to these and other conflicting reports that the value of whole grain bread in the diet has been questioned.

We have studied the effect a high content of bran in the diet had on the bioavailability of iron in pigs.

The aim in the first part of the study was to determine how a high content of bran would influence the bioavailability of iron. In the second part of the study the bioavailability of iron from cereals was compared with the bioavailability of ferrous sulfate.

Materials and methods

Animals and diets

Newborn pigs of both sexes were housed in iron-free pens. The first 7 wk they were with their mothers and suckled. In addition they were given an iron depletion diet (low iron diet) (diet I, Table 1). This diet contained 1.9% dietary fiber. At 8 and 9 wk of age they were weaned and given free access to the iron depletion diet in separate boxes (low iron diet) (diet I). Nine weeks after birth they were all anemic and then given iron repletion diets (iron rich diets). In the *first experiment* one group was fed diet II (group A, consisting of seven animals) and the other group diet III (group B, consisting of six animals). These diets contained almost the same amount of iron per kg feed. In diet II, which contained 7% bran, about 60% of the iron was derived from the bran and 40% of iron came from added ferrous sulfate. The dietary fiber content in diet II was 5.3%. In diet III, 80% of the iron came from ferrous sulfate and the dietary fiber content was 1.8%.

In the *second experiment* the pigs were after 9 wk given either diet III (group C, consisting of three animals) or diet IV (group D, consisting of four animals). Diet IV contained 15.2% dietary fiber. After dividing the pigs into these groups, group C got their additional iron from ferrous sulfate (as group B) while group D got all of their

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Received March 16, 1982.

Accepted for publication June 29, 1982.

TABLE 1
Composition of experimental diets

Ingredients	Depletion diet	Group of pigs			
		A	B and C	D	
		Repletion diets			
	I	II	III	IV	
	%	%	%	%	
Dry skimmed milk	25.0	15.0	15.0	15.0	
White wheat flour*	60.0	52.0	58.2	5.0	
Soy flour	3.0	17.0	17.0	17.0	
Barley flour†				33.2	
Sucrose	6.0	6.0	6.0	6.0	
Yeast	2.0				
Bran‡		7.0		20.0	
Salt (NaCl)	0.5	0.5	0.3	0.3	
Minerals§	3.2	3.2	3.2	3.2	
Vitamins	0.3	0.3	0.3	0.3	
Iron (mg/kg fodder)	17.0	111.0	102.0	98.0**	

* Extraction rate 78%, with dietary fiber content 3.2%.

† Extraction rate 79%, with dietary fiber content 13.6%.

‡ Bran dietary fiber content 52.8%.

§ Mineral content in the different diets are shown in Table 2.

|| 44 mg iron from FeSO_4 , 67 mg from cereals.

¶ 80 mg iron from FeSO_4 .

** All iron from cereals.

iron from bran and other cereals. Group D got 20% bran in the diet. Also in this experiment the amount of iron given was the same for the two groups.

Methods

Venous blood samples were taken each week for 7 wk. Hb concentrations were determined by the cyanmethemoglobin method (9). Serum iron concentrations were determined by an automated micromethod (10). Biological availability of iron was assessed using the hemoglobin repletion test (11).

Statistical treatment

Data from individual animals were used to calculate regression response on total iron intake in the regeneration period. Both Hb and serum-iron were regressed against weight. For statistical information we also used a fuzzy objective function clustering method. (12)

Chemical measurements

Iron content of bran and the rest of the diet was determined by atomic absorption spectrophotometry, after dry ashing at 550°C to constant weight, using Perkin Elmer 460 and dissolved in a mixture of concentrated HNO_3 and HCl (1:2).

Dietary fiber

The method used to determine dietary fiber content was based on Hellendoorn's enzymatic method (13, 14). The in vitro enzyme digestion was carried out with

pepsin at pH 1.5 for 1 h, and pancreatin at pH 6.8 for 1 h, thus simulating the conditions in the human gastrointestinal tract. Insoluble fiber components were recovered by filtration and soluble components by ethanol precipitation followed by filtration, as indicated in the procedure by Asp et al (14). With this method the removal of starch is practically complete (14). Values given are corrected for residual undigestible protein (Kjeldahl $n = 6.25$) and ash associated with the fiber.

Results

At the time of grouping (9 wk after birth) all the pigs were extremely anemic, nearly yellow in color. The Hb concentration was at that time about 5 g/100 ml (normal value 10 to 12 g/100 ml). The growth in the iron depletion phase was slower than normal, and at the time of grouping, the weights of the pigs were about 10 to 12 kg (normal 15 to 16 kg).

Figure 1 shows the increase in hemoglobin for the two groups (A and B) after iron had been added to the diet. In about 6 wk the Hb concentration had increased to about 10 to 12 g/100 ml which is normal for pigs.

Group A, receiving 7% bran in the diet, and about 60% of the iron from bran and 40% from ferrous sulfate, showed no significant difference in the increase of Hb concentration from group B, receiving no bran and nearly all their iron from ferrous sulfate.

After the first 9 wk with very little iron in

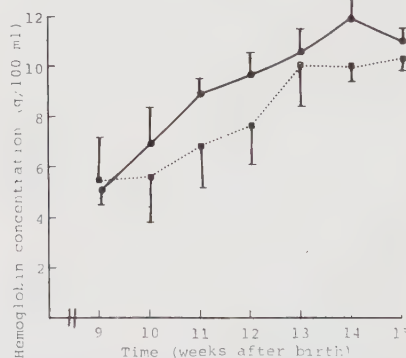


FIG. 1. Increase in Hb in iron depleted pigs after addition of iron to the diet. ●—● group A, getting 60% iron from cereals and 40% from FeSO_4 and 7% bran in the diet. ■·····■ group B, getting 80% of the iron from FeSO_4 , and no bran in the diet. Points are mean values and ± 1 SD represented by vertical bars.

the diet, all the pigs had reached pathological value of serum iron, around 2 to 3 $\mu\text{mol/l}$. After 5 to 6 wk on the repletion diet the serum iron values had increased to normal values, close to 40 $\mu\text{mol/l}$ (Fig 2), and there were no significant differences between group A and group B. Hb values for experiment 1, however, could indicate a trend toward higher Hb values for the bran diet group. The weight gains for the two groups in the repletion period were identical and showed no reduction compared with normal pigs.

This experiment showed that with moderate amounts of bran in the diet, there was no decreased bioavailability of total iron. There is a possibility, however, that all the absorbed iron could come from ferrous sulfate, even in group A. In experiment number two we therefore wanted to see if bran could be the only source of iron.

As in the first experiment, the pigs reached an anemic state with Hb values, around 5 g/100 ml after 9 wk, as shown in Figure 3. Group C which was fed the same diet as

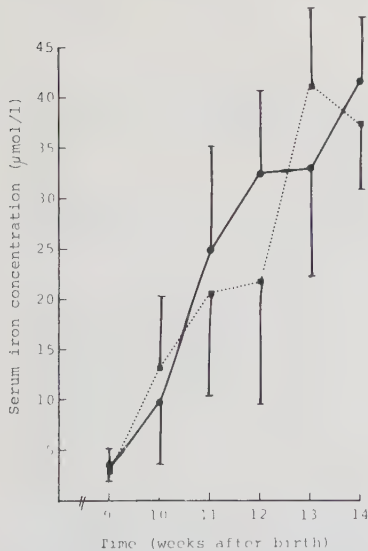


FIG. 2. Increase in serum iron in iron depleted pigs after addition of iron to the diet. ●—● group A, getting 60% iron from cereals and 40% from FeSO_4 and 7% bran in the diet. ■....■ group B, getting 80% of the iron from FeSO_4 and no bran in the diet. Points are mean values and ± 1 SD represented by vertical bars.

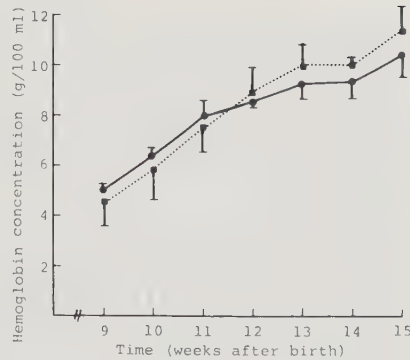


FIG. 3. Increase in hemoglobin in iron depleted pigs after addition of iron to the diet. ●—● group D, getting all iron from cereals, and 20% bran in the diet. ■....■ group C, getting 80% of the iron from FeSO_4 and no bran in the diet. Points are mean values and ± 1 SD represented by vertical bars.

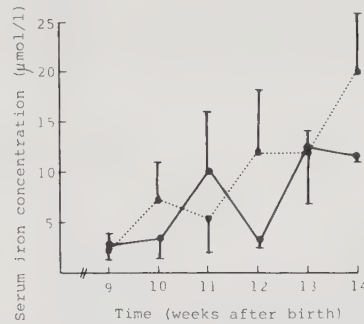


FIG. 4. Increase in serum iron in iron depleted pigs after addition of iron to the diet. ●—● group D, getting all iron from cereals and 20% bran in the diet. ■....■ group C, getting 80% of the iron from FeSO_4 and no bran in the diet. Points are mean values and ± 1 SD represented by vertical bars.

group B in the first experiment, received no bran, and nearly 80% of the iron from ferrous sulfate. Group D, however received 20% bran and almost all the iron from cereals. As shown in Figure 4, there were no significant differences between these two groups, with respect to increase in Hb values. After 9 wk with a low iron content in the diet the serum iron concentrations for the pigs in this experiment were also pathological (2 to 3 $\mu\text{mol/l}$). When adding iron to the diet, either as ferrous sulfate or as native iron from wheat bran, the

serum iron increased. There were no significant differences between the two groups; both reached a level of 10 to 20 $\mu\text{mol/l}$, which is, however, below the normal value. While the pigs in the first experiment did reach a normal serum iron level of 40 $\mu\text{mol/l}$, the animals in the second experiment never reached higher values than 20 $\mu\text{mol/l}$. We do not have any explanation for this.

As in experiment 1, body weight gains in the iron repletion period were identical for the two groups (group C and D, Fig 5), and showed no reduction compared with normal pigs (Table 2). In 6 wk, weight had increased with an average of 20 kg for each pig, which is above normal gain (Table 3).

Discussion

The aim of this study was to investigate the influence of wheat bran on iron availability.

Bran has not only a high content of iron, but also of dietary fiber and phytic acid. It has been claimed that bran could inhibit iron absorption. Bjørn-Rasmussen (4) found that

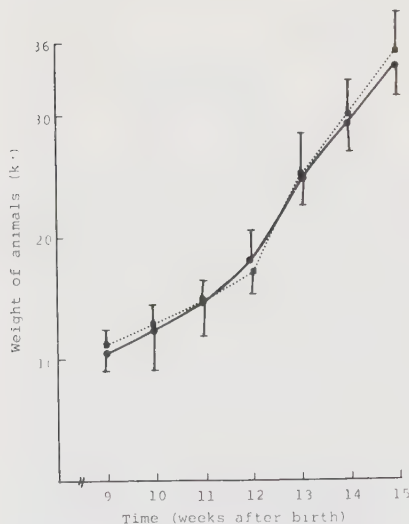


FIG. 5. Weight gain in iron depleted pigs after addition of iron to the diet. ●—● group D, getting all iron from cereals, and 20% bran in the diet. ■.....■ group C, getting 80% of the iron from FeSO_4 and no bran in the diet. Points are mean values ± 1 SD represented by vertical bars.

TABLE 2
Mineral composition of the mineral mixes (g/kg mineral mix)

Mineral	Depletion diet and diet IV	Diet II	Diet III
Ca	482.0	480.6	479.5
P	320.0	320.0	320.0
Co	0.2	0.2	0.2
Fe		1.4	2.5
Ni	90.0	90.0	90.0
Cu	1.4	1.4	1.4
Mn	2.5	2.5	2.5
J	0.1	0.1	0.1
Zn	9.8	9.8	9.8
Cl	94.0	94.0	94.0

TABLE 3
Wt gain in the iron depleted pigs after addition of iron to the diet (body wt in kg ± 2 SD)

Wk after birth	Group C (80% of iron from FeSO_4 and no bran in the diet)	Group D (all iron from cereals and 20% bran in the diet)
2	1.9 $\pm$ 0.2	2.0 $\pm$ 0.4
9	11.3 $\pm$ 0.9	10.7 $\pm$ 2.0
10	12.6 $\pm$ 1.6	12.3 $\pm$ 3.2
11	15.3 $\pm$ 1.5	14.7 $\pm$ 2.9
12	17.3 $\pm$ 2.2	18.0 $\pm$ 2.6
13	25.5 $\pm$ 3.1	25.0 $\pm$ 2.6
14	30.0 $\pm$ 2.9	29.3 $\pm$ 2.8
15	35.5 $\pm$ 3.2	34.2 $\pm$ 2.6

addition of 7% bran to white bread decreased iron absorption in humans by a factor of two. This was ascribed to the content of phytic acid in the bran.

Studies on rats, however, have shown a good bioavailability of iron in whole grain products with a high content of phytic acid (15–18).

Rats have an intestinal phytase (19, 20), which may explain why rats can utilize the iron in phytate-rich cereals. Phytase activity has also been reported in the intestinal mucosa of humans (21). It has been questioned if the phytase activity has any influence on the iron absorption in either humans or rats. (7).

Thus in the presence of bran there seem to be differences in iron absorption between humans and rats. However, all the experiments on rats are long-term, done on anemic animals and the iron absorption is measured by regeneration. The human studies, on the other hand, are short-term experiments on normal individuals with probably satisfactory iron stores.

We wanted to make a long-term experi-

ment with a model more similar to humans than rats. Piglets are suitable for experimentation because of their rapid growth, and because their intestinal tracts are more like humans than rats. For these reasons young pigs were chosen as experimental animals.

In our experiments wheat bran did not seem to have inhibitory effect on iron absorption, even when 20% bran was included in the diet. It appeared that iron from bran was as bioavailable as ferrous sulfate when pigs were repleted from an anemic state.

Factors such as ascorbic acid and chelators such as animal proteins (22, 23), are known to promote iron absorption. These dietary components are not present in the added bran. Dietary fiber and phytic acid do not seem to interfere with bioavailability of iron in the current experiments. It has been claimed by Cook et al (24) that soy flour may inhibit iron absorption. In our experiment the same amount of soy flour was present in all the repletion diets. If there is an inhibitory effect of this food component it should be to the same extent in both groups in the two experiments.

Morris and Ellis (7) have pointed out that naturally occurring iron in wheat is predominantly in the form of monoferric phytate, which seems to be a relatively available form of dietary iron.

We have found by isolating insoluble and soluble cereal fiber components (14), that almost all ash binding capacity was confined to the small, soluble fraction in the fiber. This fraction also contains most of the phytic acid (25). As the condition used in these *in vitro* experiments, the insoluble fiber in wheat bran did not seem to bind mineral.

It is, however, important to bear in mind that the chemical complexes may be different after enzymatic action (27) and even if the soluble fraction may be high in "ash," the mineral distribution in bran itself might be different.

Studies performed by Simpson et al (26) in which dephytinized bran was separated into a soluble phosphate-rich fraction and an insoluble high-fiber fraction, indicate that the soluble fraction was more inhibitory than the insoluble fraction. Which factors in the soluble fraction are responsible for the inhibitory effect is still a matter of discussion. Binding to dephytinized bran, however, could be dif-

ferent than in untreated bran, where phytic acid is known to have a binding capacity to minerals.

In conclusion, the bioavailability of iron for anemic piglets was similar in diets containing up to 20% wheat bran and most of the iron from that bran, as in diets containing similar amounts of iron mainly from ferrous sulfate. This result is in contrast to several human studies showing inhibition at least of the relative iron absorption by bran. Although species differences cannot be excluded, the long-term feedings of diets in our experiments and possibly the anemic state of the animals seem to be a more probable explanation for these different results. 

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VIII

TRACE ELEMENTS IN SERUM AND URINE OF DIABETES PATIENTS
GIVEN BREAD ENRICHED WITH WHEAT BRAN OR GUAR GUM

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Element Metabolism in Man and Animals, Aberdeen, Scotland, 1984

ABSTRACT

Twenty-eight diabetic patients, receiving insulin twice daily, went through 3 dietary periods, each lasting for 3 months. In the first (control) period, all patients used a low-fiber diet, whereas in the subsequent two (test) periods, the bread rations were enriched with either wheat bran or guar gum in a cross-over pattern. The mean daily supplementation per patient was 33 g of bran and 29 g of guar. During the test and control periods trace metal analyses were carried out using atomic absorption spectrophotometry.

The fiber treatment with wheat bran or guar gum reduced the mean blood glucose to 9.9 ± 2.6 or 9.9 ± 2.8 mmol/l, respectively, from a pretreatment value of 11.8 ± 3.5 mmol/l. At the start of the test period the serum levels of Cu, Zn, Se, and Fe, respectively, were 15.6 ± 2.5 , 16.1 ± 1.6 , 1.46 ± 0.23 and 17.1 ± 4.1 μ mol/l. No changes were observed in the serum levels of trace elements during the fiber enrichment periods. Furthermore, no changes were seen in the serum levels of Ca or Mg, although the urinary excretion of Ca showed a decreasing tendency during the test periods.

Our study indicates that wheat bran as well as guar gum can reduce blood glucose in diabetics without significantly interfering with the trace element metabolism.

Key words: Zinc, magnesium, calcium, selenium, dietary fibers, diabetes.

Several previous studies have shown that addition of gelforming fibers, such as guar gum, to the diet of diabetic patients improves their metabolic control, as judged from postprandial blood glucose values and urinary glucose excretion (Vahouny 1982). Similar studies with wheat bran are not conclusive. Most studies have been of short duration and done with a small number of test persons, mainly with type II diabetics.

The possibility that fiber supplementation may lead to side effects has been discussed by many authors, because it is reported that some diets high in fiber have been associated with a high incidence of mineral imbalance (Van Soest 1976).

Wholemeal bread, rich in fiber and phytate, which is consumed in Iran and Middle Eastern countries, may interfere with the absorption of calcium, magnesium, iron, zinc, and phosphorus in man (Reinhold et al 1976).

Studies using wheat bran or guar gum are, however, not conclusive in this respect.

The present study was undertaken to compare the metabolic effects of long-term addition of guar gum and wheat bran to the diet of insulin-dependent diabetic patients.

MATERIALS AND METHODS

Thirty-three insulin-dependent diabetics were initially recruited. Five patients dropped out during the normal period. Relevant patient data for the remaining 28 patients are shown in table 1. There were no leftouts during the treatment periods.

None of the patients had persistent proteinuria or major neuropathy, while a few had reinopathy that had needed laser treatment.

During the whole study all patients consulted their ordinary doctors as usual, and no results were given to patients or doctors before the study ended. With the exception of the fiber supplementation, no efforts were done to improve the diabetic control.

Every patient went through 3 dietary periods, each lasting for 3 months. The first was a normal period when the patients followed their ordinary dietary habits. In Norway, the intake of bread is relatively high, because only one hot meal is eaten daily. The patients used an ordinary, low fiber bread and

the mean intake was 210 g/day (range: 120 - 390 g/day). The fiber content in this diet is estimated to about 15 to 20 g/day.

Then their bread rations were enriched with guar gum in one period (guar period), and bran in another (bran period). The fibers were baked into wheat-flour bread and the diabetics continued to eat their usual amount of bread. Mean guar supplementation was 29 g/day (range: 17 - 55 g/day) and mean bran supplementation was 33 g/day (range: 19 - 62 g/day). The enrichment followed a cross-over pattern.

Blood glucose was measured in the fasting state and 2 hours after each meal, one day every week at home with a filter paper method.

Urine was collected for 24 hours, with fortnight intervals, and analyzed for glucose (glucose-DH, Merck, F.R.G.), calcium, inorganic phosphate, creatinine, zinc, magnesium, and selenium. Parameters measured monthly at the outpatient clinic included HbA₁ and serum concentrations of calcium, inorganic phosphate, iron, magnesium, copper, zinc, and selenium. At the same visit they were given their monthly bread rations.

Selenium was analyzed with atomic absorption spectrophotometry using a graphite furnace (Saeed et al 1979). The other trace metals were determined with atomic absorption using ordinary flame atomization. Other laboratory measurements were carried out by routine analytical techniques. The statistical evaluation of the results made use of Student's test.

RESULTS

Mean blood glucose values decreased from 11.8 ± 3.5 mmol/l (mean $\pm$ SD) in the normal period, to 9.9 ± 2.8 mmol/l in the guar period, and 9.9 ± 2.6 mmol/l in the bran period.

The daily urinary excretion of glucose showed a decreasing tendency during the fiber periods (table 2).

Mean HbA₁ decreased from 10.5 ± 2.1 per cent in the normal period to 9.7 ± 1.6 per cent at the end of the guar period, and 9.9 ± 1.2 per cent at the end of the bran period.

No changes were measured in the serum concentrations of calcium, inorganic phosphate, magnesium, iron, copper, zinc or selenium (table 3).

The urinary excretion of calcium was slightly lowered during the treatment with wheat bran, whereas the excretion rates of inorganic phosphate, magnesium, zinc (table 4), and selenium were essentially unchanged.

DISCUSSION

It appears clearly from our study that dietary supplementation with guar gum or wheat bran can improve the glucose metabolism in patients with diabetes. This is consistent with results from other studies (Jenkins 1980), although they have not carried out detailed studies on the effects of wheat bran after long-term supplementation.

In our patients, the fiber supplementation did not cause any changes in serum concentrations or urinary excretion of trace elements or minerals, with the exception that wheat bran reduced the urinary calcium excretion to around 80 per cent of the pretreatment values. In some previous studies, dietary fibers have been shown to act as weak cation exchange resins, binding Zn, Ca, and Fe, making them unavailable for absorption (Davies et al 1976), whereas others have failed to confirm these findings. Apparently, the reported effects of dietary fibers on trace elements and mineral balances in man are inconsistent (Vahouny 1982). Since the fecal excretion of inorganic elements was not measured in our patients, quantitative data on the absorption of the elements cannot be given here. However, it appears from our data on serum concentrations and urinary excretion that the fiber-induced changes in the metabolism of the inorganic elements are of negligible physiological significance.

Controversial results in different studies as regards the effects on mineral absorption may be due to several variables: Differences in the nature and level of dietary fiber components, presence of interfering substances such as phytate or oxalates, and differences in levels of ingested micro-nutrients.

In conclusion our study has shown that wheat bran as well as guar gum can improve the glucose metabolism of diabetics, without significantly affecting the metabolism of trace elements or minerals.

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TABLE 1

Relevant patient data

	Mean	Range
Age	27	17 - 54
Diabetes duration (years)	15	4 - 26
Body weight at start (kg)	69.4 [*]	55.5 - 94.5
Insulin dose (IU) ^{**}		
morning	31	12 - 42
evening	21	12 - 42

* No significant changes were measured in body weight during the study.

** The insulin doses were not changed during the study.

TABLE 2

Effects of fiber supplementation on glucose metabolism

Parameter (Mean $\pm$ SD)	Normal period	Guar period	Bran period
Fasting blood glucose (mmol/l)	11.4 $\pm$ 3.6	10.8 $\pm$ 2.8	10.5 $\pm$ 3.3
Postprandial blood glucose (mmol/l)	12.0 $\pm$ 3.8	9.7 $\pm$ 2.8*	9.7 $\pm$ 2.7*
Mean blood glucose (mmol/l)	11.8 $\pm$ 3.5	9.9 $\pm$ 2.8*	9.9 $\pm$ 2.6**
Daily urinary glucose excretion (mmol per mmol creatinine)	22.3 $\pm$ 24.4	15.7 $\pm$ 16.3	16.4 $\pm$ 14.1
HbA ₁ (last value in each period, in per cent of total HbA)	10.5 $\pm$ 2.1	9.7 $\pm$ 1.6***	9.9 $\pm$ 1.2

* P < 0.01

** P < 0.025

*** P < 0.05

TABLE 3

Serum concentrations (mean $\pm$ SD) of calcium, inorganic phosphate (PO_4), magnesium, iron, zinc, copper, and selenium at the end of the fiber supplementation periods

	Control period	Guar period	Bran period
Ca, mmol/l	2.29 $\pm$ 0.06	2.28 $\pm$ 0.06	2.30 $\pm$ 0.05
PO_4 , mmol/l	1.25 $\pm$ 0.13	1.15 $\pm$ 0.14	1.20 $\pm$ 0.15
Mg, mmol/l	0.71 $\pm$ 0.05	0.71 $\pm$ 0.05	0.72 $\pm$ 0.07
Fe, $\mu\text{mol/l}$	17.1 $\pm$ 4.1	17.0 $\pm$ 6.0	17.3 $\pm$ 5.9
Zn, $\mu\text{mol/l}$	16.1 $\pm$ 1.6	16.5 $\pm$ 2.2	16.6 $\pm$ 2.5
Cu, $\mu\text{mol/l}$	15.6 $\pm$ 2.5	15.2 $\pm$ 2.8	15.2 $\pm$ 3.1
Se, $\mu\text{mol/l}$	1.46 $\pm$ 0.23	1.48 $\pm$ 0.22	1.49 $\pm$ 0.22

TABLE 4

Effects of fiber supplementation on urinary excretion of Ca, PO₄, Mg, and Zn, expressed as mmol of the inorganic element per mmol of creatinine excreted

	Normal period	Guar period	Bran period
Ca	0.48 ± 0.17	0.43 ± 0.18	0.38 ± 0.13*
PO ₄	2.95 ± 0.72	3.02 ± 0.58	3.10 ± 0.48
Mg (n = 24)	0.38 ± 0.15	0.35 ± 0.15	0.39 ± 0.20
Znx10 ³ (n = 24)	1.2 ± 0.7	1.2 ± 0.7	1.1 ± 0.6

* P < 0.01

